1.C.44 The Plant Thionin (PT) Family

Most mature plant thionins are 45-47aas in length, but their precursors are larger (70-80aas). There are two subgroups, the 8 cysteine (4 disulfide bridge) and the six cysteine (3 disulfide bridge) thionins. Thionin sequences are highly divergent, and only the six cysteines at positions 3, 4, 10, 27, 33 and 41 as well as an arginine at position 10 and an aromatic residue at position 13 are well conserved. Three-dimensional analyses reveal a very similar fold for all thionins (an L shape with the long arm formed by two antiparallel α-helices and the short arm by two antiparallel β-strands). There amphipathic structures inhibit the growth of bacteria and fungi. They form pores in the membranes of these cells as well as of yeast, animal cells and plant protoplasts. Ions transported include H^+, Ca^{2+}, K^+, and $H_2PO_4^-$. Organic substances (amino acids, nucleobases, sugars) are also released via these channels.

The generalized transport reaction is:

small molecules (in) $\rightleftharpoons$ small molecules (out)

References:

Broekaert, W.F., B.P.A. Cammue, M.F.C. De Bolle, K. Thevissen, G.W. De Samblanx and R.W. Osborn (1997). Antimicrobial peptides from plants. Crit. Rev. Plant. Sci. 16: 297-323.

Garcia-Olmedo, F., A. Molina, J.M. Alamillo and P. Rodriguez-Palenzuela (1998). Plant defense peptides. Biopolymers. 479-491.

Examples:

TC#	Name	Organismal Type	Example
1.C.44.1.1	**β-purothionin (A-I) precursor**	**Plants**	**β-purothionin precursor of *Triticum aestivum***
1.C.44.1.2	**Viscotoxin B precursor**	**Plants**	**Viscotoxin B precursor of *Viscum album***

Format for Printing

1.C.44 The Plant Thionin (PT) Family

Most mature plant thionins are 45-47aas in length, but their precursors are larger (70-80aas). There are two subgroups, the 8 cysteine (4 disulfide bridge) and the six cysteine (3 disulfide bridge) thionins. Thionin sequences are highly divergent, and only the six cysteines at positions 3, 4, 10, 27, 33 and 41 as well as an arginine at position 10 and an aromatic residue at position 13 are well conserved. Three-dimensional analyses reveal a very similar fold for all thionins (an L shape with the long arm formed by two antiparallel α-helices and the short arm by two antiparallel β-strands). There amphipathic structures inhibit the growth of bacteria and fungi. They form pores in the membranes of these cells as well as of yeast, animal cells and plant protoplasts. Ions transported include H^+, Ca^{2+}, K^+, and $H_2PO_4^-$. Organic substances (amino acids, nucleobases, sugars) are also released via these channels.

The generalized transport reaction is:

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View Proteins

Broekaert, W.F., B.P.A. Cammue, M.F.C. De Bolle, K. Thevissen, G.W. De Samblanx and R.W. Osborn (1997). Antimicrobial peptides from plants. Crit. Rev. Plant. Sci. 16: 297-323.

Garcia-Olmedo, F., A. Molina, J.M. Alamillo and P. Rodriguez-Palenzuela (1998). Plant defense peptides. Biopolymers. 479-491.

9 Genuine Article#: 353AL Number of References: 42
Title: NMR structural determination of viscotoxin A3 from Viscum album L.
Author(s): Romagnoli S; Ugolini R; Fogolari F; Schaller G; Urech K; Giannattasio M; Ragona L; Molinari H (REPRINT)
Corporate Source: DIPARTIMENTO SCI & TECNOL,CA VIGNAL 1, STRADA GRAZIE/I-37134 VERONA//ITALY/ (REPRINT); DIPARTIMENTO SCI & TECNOL,/I-37134 VERONA//ITALY/; HISCHIA INST,VEREIN KREBSFORSCH/CH-4144 ARLESHEIM//SWITZERLAND/; UNIV NAPLES,FAC AGR, DIPARTIMENTO ARBORICOLTURA BOT & PATOL VEGETALE/I-80100 NAPLES//ITALY/; IST CHIM MACROMOL,LAB NMR/I-20131 MILAN//ITALY/
Journal: BIOCHEMICAL JOURNAL, 2000, V350, 2 (SEP 1), P569-577
ISSN: 0264-6021 Publication date: 20000901
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND
Language: English Document Type: ARTICLE
Geographic Location: ITALY; SWITZERLAND
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: The high-resolution three-dimensional structure of the plant toxin viscotoxin A3, from Viscum album L., has been determined in solution by H-1 NMR spectroscopy at pH 3.6 and 12 degrees C (the structure has been deposited in the Protein Data Bank under the id. code 1EDO). Experimentally derived restraints including 734 interproton distances from nuclear Overhauser effect measurements, 22 hydrogen bonds, 32 phi angle restraints from J coupling measurements, together with three disulphide bridge constraints were used as input in restrained molecular dynamics, followed by minimization, using DYANA and Discover. Backbone and heavy atom root-mean-square deviations were 0.47+/-0.11 Angstrom (1 Angstrom = 10(-10) m) and 0.85+/-0.13 Angstrom respectively. Viscotoxin A3 consists of two **alpha** -helices connected by a turn and a short stretch of antiparallel **beta** -sheet. This fold is similar to that found in other **thionins** , such as crambin, hordothionin- **alpha** and - **beta** , phoratoxin A and purothionin- **alpha** , and - **beta** . The difference in the observed biological activity for **thionins** of known structure is discussed in terms of the differences in the calculated surface potential distribution, playing an important role in their function through disruption of cell membranes. In addition, the possible role in DNA binding of the helix-turn-helix motif of viscotoxin A3 is discussed.
Descriptors--Author Keywords: electrostatic calculation ; nuclear magnetic resonance ; structure determination ; **thionins** ; viscotoxins
Identifiers--KeyWord Plus(R): NUCLEAR-MAGNETIC-RESONANCE; 3-DIMENSIONAL STRUCTURE; SECONDARY STRUCTURE; **PYRULARIA THIONIN** ; RESPONSES; DYNAMICS; PROGRAM; BARLEY; **ALPHA**; ELECTROSTATICS
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3/9/9 (Item 4 from file: 34)

08354396 Genuine Article#: 275TQ Number of References: 33

Title: The cytotoxic plant protein, beta -purothionin, forms ion channels in lipid membranes

Author(s): Hughes P; Dennis E; Whitecross M; Llewellyn D (REPRINT) ; Gage P
Corporate Source: CSIRO,PLANT IND, CLUNIES ROSS ST/CANBERRA/ACT 2601/AUSTRALIA/ (REPRINT); CSIRO,PLANT IND/CANBERRA/ACT 2601/AUSTRALIA/ ; AUSTRALIAN NATL UNIV,DIV BOT & ZOOL/CANBERRA/ACT 0200/AUSTRALIA/; AUSTRALIAN NATL UNIV,JOHN CURTIN SCH MED RES, MEMBRANE BIOL PROGRAM/CANBERRA/ACT 2601/AUSTRALIA/

Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 2000, V275, N2 (JAN 14), P823-827
ISSN: 0021-9258 Publication date: 20000114
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Geographic Location: AUSTRALIA
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY

Abstract: **Thionins** are small cysteine-containing, amphipathic plant proteins found in seeds and vegetative tissues of a number of plant genera, Many of them have been shown to be toxic to microorganisms such as fungi, yeast, and bacteria and also to mammalian cells. It has been suggested that **thionins** are present in seeds to protect them, and the germinating seedling, from attack by phytopathogenic microorganisms, but the mechanism by which they kill cells remains unclear. Using electrophysiological measurements, we have shown that **beta** -purothionin from wheat flour can form cation-selective ion channels in artificial lipid bilayer membranes and in the plasmalemma of rat hippocampal neurons. We suggest that the generalized toxicity of **thionins** is due to their ability to generate ion channels in cell membranes, resulting in the dissipation of ion concentration gradients essential for the maintenance of cellular homeostasis.

Identifiers--KeyWord Plus(R): CULTURED HIPPOCAMPAL-NEURONS; **PYRULARIA THIONIN** ; **ALPHA - THIONIN** ; RESPONSES; CARDIOTOXIN; RESISTANCE; MELITTIN; BARLEY

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THEVISSEN K, 1996, V271, P15018, J BIOL CHEM
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3/9/11 (Item 6 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05968861 Genuine Article#: XL001 Number of References: 34

Title: Differential effects of five types of antipathogenic plant peptides on model membranes

Author(s): Caaveiro JMM; Molina A; GonzalezManas JM; RodriguezPalenzuela P; GarciaOlmedo F; Goni FM (REPRINT)
Corporate Source: UNIV PAIS VASCO,CSIC, DEPT BIOQUIM, GRP BIOMEMBRANAS, UNIDAD ASOCIADA, APTDO 644/E-48080 BILBAO//SPAIN/ (REPRINT); UNIV PAIS VASCO,CSIC, DEPT BIOQUIM, GRP BIOMEMBRANAS, UNIDAD ASOCIADA/E-48080 BILBAO//SPAIN/; UPM,ETS INGENIEROS AGRON, LAB BIOQUIM & BIOL MOL/MADRID 28040//SPAIN/

Journal: FEBS LETTERS, 1997, V410, N2-3 (JUN 30), P338-342
ISSN: 0014-5793 Publication date: 19970630
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Geographic Location: SPAIN
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOPHYSICS; BIOCHEMISTRY & MOLECULAR BIOLOGY

Abstract: The effects of five antipathogenic plant peptides, wheat **alpha - thionin** , potato PTH1 defensin, barley LTP2 lipid transfer protein, and potato tuber DL1 and DL2 defensins, have been tested against phospholipid vesicles (liposomes). Wheat **thionin** very actively induces aggregation and leakage of negatively charged vesicles. LTP2 displays the same activities, although to a limited extent. Under certain conditions PTH1 and DL2 induce vesicle aggregation, but not leakage. Potato defensin DL1 failed to show any effect on liposomes. The same peptides have been assayed against a plant pathogenic bacterium, both the membrane-active and -inactive compounds having efficient antibacterial action. (C) 1997 Federation of European Biochemical Societies.

Descriptors--Author Keywords: **thionin** ; defensin ; lipid transfer protein ; plant pathogen ; biomembrane
Identifiers--KeyWord Plus(R): LIPID TRANSFER PROTEINS; **PYRULARIA THIONIN** ; VESICLE CONTENTS; FUSION; PATHOGENS; BACTERIAL; LEAKAGE; WHEAT
Research Fronts: 95-1783 001 (PHOSPHATIDYLCHOLINE CHOLESTEROL LIPOSOMES; MEMBRANE INTERACTIONS; WOOL DYEING)

95-6888 001 (PHOSPHATIDYLCHOLINE LIPOSOMES; LIPID BILAYERS; LARGE UNILAMELLAR VESICLES; PHOSPHOLIPASE A(2); MODEL SYSTEMS)

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THEEVISSEN K, 1996, V271, P15018, J BIOL CHEM
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3/9/13 (Item 8 from file: 34)

04910953 Genuine Article#: UT106 Number of References: 49

Title: FUNGAL MEMBRANE RESPONSES INDUCED BY PLANT DEFENSINS AND THIONINS

Author(s): THEVISSEN K; GHAZI A; DESAMBLANX GW; BROWNLEE C; OSBORN RW; BROEKAERT WF

Corporate Source: KATHOLIEKE UNIV LEUVEN,FA JANSSENS LAB GENET,WILLEM CROYLAAN 42/B-3001 HEVERLEE//BELGIUM/; KATHOLIEKE UNIV LEUVEN,FA JANSSENS LAB GENET/B-3001 HEVERLEE//BELGIUM/; UNIV PARIS 11,URA CNRS 1116,LAB BIOMEMBRANES/F-91405 ORSAY//FRANCE/; MARINE BIOL ASSOC UNITED KINGDOM LAB/PLYMOUTH PL1 2PB/DEVON/ENGLAND/; ZENECA AGROCHEM,JEALOTTS HILL RES STN/BRACKNELLRG42 6ET/BERKS/ENGLAND/

Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1996, V271, N25 (JUN 21), P 15018-15025

ISSN: 0021-9258

Language: ENGLISH Document Type: ARTICLE

Geographic Location: BELGIUM; FRANCE; ENGLAND

Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences

Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY

Abstract: Treatment of hyphae of Neurospora crassa with anti fungal plant defensins, i.e. Rs-AFP2 and Dm-AMP1 isolated from radish and dahlia seed, respectively, induced a rapid K+ efflux, Ca2+ uptake, and alkalinization of the incubation medium, The Rs-AFP2-induced alkalinization of the incubation medium could be inhibited with G-protein inhibitors. **alpha** -Hordothionin, an antifungal **thionin** from barley seed, caused a sustained increased Ca2+ uptake at

subinhibitory concentrations but only a transient increased uptake at inhibitory concentrations, **alpha** -Hordothionin also caused increased K+ efflux and alkalinization of the medium, but these fluxes occurred more rapidly compared to those caused by plant defensins. Furthermore, **alpha** -hordothionin caused permeabilization of fungal hyphae to the non-metabolite **alpha** aminoisobutyric acid and, in addition, altered the electrical properties of artificial lipid bilayers, consistently leading to rupture of the lipid bilayers, The plant defensins did not form ion permeable pores in artificial membranes and did not exhibit substantial hyphal membrane permeabilization activity, Our results are consistent with the notion that **thionins** inhibit fungal growth as a result of direct protein-membrane interactions, whereas plant defensins might act via a different, possibly receptor-mediated, mechanism.

Identifiers--KeyWords Plus: CULTURED TOBACCO CELLS; ACTIVATED CHANNELS; **PYRULARIA THIONIN** ; CRYSTAL-STRUCTURE; ESCHERICHIA-COLI; INSECT DEFENSIN; MAMMALIAN-CELLS; ION CHANNELS; TIP GROWTH; PEPTIDES

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     $0.07  Estimated cost File467
            OneSearch, 26 files,  0.320 DialUnits FileOS
     $0.26  TELNET
    $41.78  Estimated cost this search
    $41.78  Estimated total session cost   0.320 DialUnits


### Status: Signed Off. (1 minutes)
```

...extraction buffer containing 50 mM TRIS-chloride, pH
81 100 mM sodium chloride, 10 mM **EDTA** , pH 81 1OW sodium dodecyl
sulfate, and 10 mM beta-mercaptoethanol (added immediately before
use...extraction buffer (10 mM sodium chloride, 10 mM TRIS-Cl, pH 9.0j, 1
mM **EDTA**), 2.5 mL phenol (saturated with TRIS buffer at pH 4.31
Fisher #BP1751), and...

...formaldehyde (Fisher #BP531), 20 mM MOPS, pH 7.01 5 mM sodium
acetate, 10 mM **EDTA** , and 0.01% bromophenol blue. RNA samples were
heated at 600C for 10 to 15...

...was
dissolved in 30 mL of 20 mM MOPS, 5 mM sodium acetate, 10 mM **EDTA** ,
pH 7.0, to which was added 37 % formaldehyde for a final
concentration of 6...

...M sodium phosphate buffer, pH
7.01 0.25 M sodium chloride, and 1 mM **EDTA** , pH 8.0, as recommended
by Bio-Rad. Probes for northern blots consisted of partial...consisting
of 89 mM TRIS-Cl, pH 8.31 89 mM borate, 1.5 mM
EDTA . The resulting endochitinase cleavage products, shown in
FIG. 16, closely matched those expected from the...

15/3,KWIC/37 (Item 6 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00754251
X-RAY GUIDED DRUG DELIVERY
ADMINISTRATION DE MEDICAMENT GUIDEE PAR RAYON X
Patent Applicant/Assignee:
VANDERBILT UNIVERSITY, Office of Technology Transfer, Suite 210, 1207 17th Avenue South, Nashville, TN 37212, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
HALLAHAN Dennis E, 4214 Estes Road, Nashville, TN 37215, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
TAYLOR Arles A Jr, Jenkins & Wilson, P.A., University Tower, Suite 1400, 3100 Tower Boulevard, Durham, NC 27707, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 200066182 A1 20001109 (WO 0066182)
Application: WO 2000US11485 20000428 (PCT/WO US0011485)
Priority Application: US 99302456 19990429
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU CA JP US
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Filing Language: English
Fulltext Word Count: 41390

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... I m e t h a c ry I a t e,
polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, **polyethyleneglycol** , and polyaspartamide. In a preferred embodiment, the hydrophilic polymer is **polyethyleneglycol** (PEG), preferably as a PEG chain
having a molecular weight between 500-10,000 daltons...

...sodium lactate, sodium
phosphate, Tris, and N-methyl glucamine. Examples of suitable chelating agents include **EDTA** , DTPA, DTPA-BMA and salts and complexes thereof especially calcium, sodium or meglumine salts, e. g. edetate disodium, edetic acid, calcium **EDTA** .
Examples of suitable anti-oxidants include ascorbic acid, ascorbyl palmitate, cysteine, monothioglycerol, butylated hydroxyanisole, butylated...

...which aid in the Iyophilization and reconstitution processes include sodium chloride, sorbitol, mannitol, glucose and **polyethyleneglycol** .

Representative starting materials and method for the preparation of 1 0 liposomes are also disclosed...

...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macrornomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...of endothelial cell cultures was verified by staining for factor
VIII. ConfluentcellswereharvestedwithO.l%collagenaseO.01% **EDTAand** subcultured at a ...these liposomes bind to lipophilic proteins in the serum, which reduces the circulation time.
Therefore, **polyethyleneglycol** (PEG) is used to coat the drug delivery systems.

PEG prolongs circulation time (Nam, Scontaining 1 OmM **EDTA** and 0.08% NaN3is added 5x excess of freshly prepared Traut's reagent in the...

...liposomes. Prepared vesicles and thiolated protein is mixed in 10mm Hepes, 0.15M NaCl and **EDTA** pH 6 The finai concentrations for proteins and liposomes are 0.25 g/L and...

Claim
... 4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine,
vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin,
...daunorubicin, cytosine arabinoside, etoposide, 5-4 fluorouracil, melphalan,
chlorambucil, cyclophosphamide, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine,
vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinonederivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine, neocarzinostatin...group consisting of polyvinylpyrrolidone,
polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate,
polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, **polyethyleneglycol** , polyaspartamide and combinations thereof.
146. The delivery vehicle of claim 142, wherein the targeting agent...

15/3,KWIC/38 (Item 7 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

13449473 Genuine Article#: 882PC Number of References: 53
Title: Proposal for molecular mechanism of thionins **deduced from physico-chemical studies of plant toxins**
Author(s): Stec B (REPRINT) ; Markman O; Rao U; Heffron G; Henderson S; Vernon LP; Brumfeld V; Teeter MM
Corporate Source: Univ Texas,Dept Chem,500 W Univ Ave/El Paso//TX/79968 (REPRINT); Univ Texas,Dept Chem,El Paso//TX/79968; Boston Coll,Merkert Chem Ctr, Dept Chem,Chestnut Hill//MA/02467; Oak Ridge Natl Lab,Div Biol,Oak Ridge//TN/37831; Brigham Young Univ,Dept Chem,Provo//UT/84602; Weizmann Inst Sci,Dept Biochem,IL-76100 Rehovot//Israel/(bstec@utep.edu)
Journal: JOURNAL OF PEPTIDE RESEARCH, 2004, V64, N6 (DEC), P210-224
ISSN: 1397-002X Publication date: 20041200
Publisher: BLACKWELL MUNKSGAARD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK
Language: English Document Type: ARTICLE
Geographic Location: USA; Israel
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: We propose a molecular model for phospholipid membrane lysis by the ubiquitous plant toxins called **thionins** . Membrane lysis constitutes the first major effect exerted by these toxins that initiates a cascade of cytoplasmic events leading to cell death. X-ray crystallography, solution nuclear magnetic resonance (NMR) studies, small angle X-ray scattering and fluorescence spectroscopy provide evidence for the mechanism of membrane lysis. In the crystal structures of two **thionins** in the family, **alpha** (1)- and **beta** -purothionins (MW: approximately 4.8 kDa), a phosphate ion and a glycerol molecule are modeled bound to the protein. P-31 NMR experiments on the desalted toxins confirm phosphate-ion binding in solution. Evidence also comes from phospholipid partition experiments with radiolabeled toxins and with fluorescent phospholipids. This data permit a model of the phospholipid-protein complex to be built. Further, NMR experiments, one-dimensional (1D)- and two-dimensional (2D)-total correlation spectroscopy (TOCSY), carried out on the model compounds glycerol-3-phosphate (G3P) and short chain phospholipids, supported the predicted mode of phospholipid binding. The toxins' high positive charge, which renders them extremely soluble (>300 mg/mL), and the phospholipid-binding specificity suggest the toxin-membrane interaction is mediated by binding to patches of negatively charged phospholipids [phosphatidic acid (PA) or phosphatidyl serine (PS)] and their subsequent withdrawal. The formation of proteolipid complexes causes solubilization of the membrane and its lysis. The model suggests that the oligomerization may play a role in toxin's activation process and provides insight into the structural principles of protein-membrane interactions.
Descriptors--Author Keywords: mechanism of toxicity ; membrane lysis ; plant toxins ; **thionins**
Identifiers--KeyWord Plus(R): NUCLEAR-MAGNETIC-RESONANCE; SOLUTE PARTICLES IMPORTANT; **PYRULARIA THIONIN** ; 3-DIMENSIONAL STRUCTURE; ERYTHROCYTE-MEMBRANES; TERTIARY STRUCTURE; CRYSTAL-STRUCTURE; LATTICE FORMATION; PROTEIN CRAMBIN; PUROTHIONIN
Cited References:

10435746 Genuine Article#: 527DU Number of References: 62
Title: Modes of membrane interaction of a natural cysteine-rich peptide: viscotoxin A3
Author(s): Coulon A; Berkane E; Sautereau AM; Urech K; Rouge P; Lopez A (REPRINT)
Corporate Source: CNRS,Inst Pharmacol & Biol Struct, UMR 5089,205 Route Narbonne/F-31077 Toulouse 4//France/ (REPRINT); CNRS,Inst Pharmacol & Biol Struct, UMR 5089,F-31077 Toulouse 4//France/; Hiscia Inst,Verein Krebforsch,CH-4144 Arlesheim//Switzerland/
Journal: BIOCHIMICA ET BIOPHYSICA ACTA-BIOMEMBRANES, 2002, V1559, N2 (FEB 15), P145-159
ISSN: 0005-2736 Publication date: 20020215
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Geographic Location: France; Switzerland
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; BIOPHYSICS
Abstract: Among the very homologous family of **alpha** , and **beta** - **thionins** , known for their antimicrobial activity, the viscotoxin subfamily differs from other members because it is cytotoxic against tumoral cells but weakly hemolytic. We studied the interactions between the most active of these toxins, viscotoxin A3 (VA3), and model membranes made of phosphatidylcholine and phosphatidylserine (PS), the major zwitterionic and acidic phospholipids found in eukaryotic cells. Monolayer studies showed that electrostatic forces are essential for the interaction and are mainly involved in modulating the embedding of the toxin in the PS head group region. This in turn induces membrane stiffening, as shown by fluorescence polarization assays with 1,6-diphenyl-1,3,5-hexatriene and its derivatives. Moreover, vesicle permeabilization analyses showed that there are two modes of interaction, which are directly related to the stiffening effect and depend on the amount of VA3 bound to the surface of the vesicles. We propose an interaction model in which the embedding of VA3 in the membrane induces membrane defects leading to the gradual release of encapsulated dye. When the surfaces of the vesicles are saturated with the viscotoxin, complete vesicle destabilization is induced which leads to bilayer disruption, all-or-none encapsulated dye release and rearrangement of the vesicles. (C) 2002 Elsevier Science B.V. All rights reserved.
Descriptors--Author Keywords: viscotoxin ; **thionin** ; membrane ; phosphatidylserine ; release ; polarization
Identifiers--KeyWord Plus(R): VISCUM-ALBUM L; MOLECULAR-DYNAMICS SIMULATION; LARGE UNILAMELLAR VESICLES; FRIEND-ERYTHROLEUKEMIC CELLS; **PYRULARIA** - **THIONIN** ; ANTIMICROBIAL PEPTIDES; PHOSPHOLIPID-MEMBRANES; BILAYER-MEMBRANES; LIPID-MEMBRANES; CECROPIN P1
Cited References:

11/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12120265 PMID: 9421187

Mechanisms by which thionin **induces susceptibility of S49 cell membranes to extracellular phospholipase A2.**

Wilson H A; Huang W; Waldrip J B; Judd A M; Vernon L P; Bell J D
Department of Zoology, Brigham Young University, Provo, UT 84602, USA.

Biochimica et biophysica acta (NETHERLANDS) Nov 15 1997, 1349 (2) p142-56, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: GM-49710; GM; NIGMS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Whereas cells normally resist attack by PLA2, they become susceptible under certain pathological conditions. To ascertain the regulatory mechanisms that induce cellular susceptibility to PLA2, the effect of **thionin** on S49 cells was examined in the presence of PLA2. **Thionin** alone was unable to evoke hydrolysis of the lipid bilayer. Likewise, the addition of PLA2 alone caused production of only a minimal amount of free fatty acid. However, **thionin** and PLA2 together resulted in significant hydrolysis of the cell membrane. **Thionin** caused perturbation of the bilayer structure as suggested by the changes in the emission spectra of laurdan and the permeability of the membrane to propidium iodide. These changes correlated quantitatively with the susceptibility of the lipid bilayer to PLA2. Furthermore, **thionin** induced a modest increase in intracellular Ca2+. The source of this Ca2+ was the extracellular fluid since **EDTA** in the extracellular medium inhibited the Ca2+ influx. Moreover, cobalt chloride, a universal Ca2+ channel blocker, prevented the rise in intracellular Ca2+, the uptake of propidium iodide, and the susceptibility to PLA2 induced by **thionin** . In contrast, the changes in the laurdan emission caused by the **thionin** were not affected by the cobalt. Furthermore, incubation of the cells with the calcium ionophore A23187 also caused the cells to become susceptible to PLA2. We hypothesize that **thionin** causes S49 cell membranes to become susceptible to PLA2 by a Ca2+-dependent perturbation of the bilayer structure.

Tags: Research Support, U.S. Gov't, P.H.S.
Descriptors: *Phenothiazines--pharmacology--PD; *Phospholipases A --pharmacology--PD; Animals; Arachidonic Acid--metabolism--ME; Calcimycin --pharmacology--PD; Calcium--metabolism--ME; Cell Membrane--metabolism--ME; Lipid Bilayers--metabolism--ME; Lymphoma--metabolism--ME; Mice; Tumor Cells, Cultured
CAS Registry No.: 0 (Lipid Bilayers); 0 (Phenothiazines); 506-32-1 (Arachidonic Acid); 52665-69-7 (Calcimycin); 581-64-6 (thionine); 7440-70-2 (Calcium)
Enzyme No.: EC 3.1.1.- (Phospholipases A)
Record Date Created: 19980115
Record Date Completed: 19980115

11/9/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10106631 PMID: 7680580

Thionin **staining of paraffin and plastic embedded sections of cartilage.**

Bulstra S K; Drukker J; Kuijer R; Buurman W A; van der Linden A J
Department of Orthopaedic Surgery, University Hospital Maastricht, State University of Limburg, The Netherlands.

Biotechnic & histochemistry - official publication of the Biological Stain Commission (UNITED STATES) Jan 1993, 68 (1) p20-8, ISSN 1052-0295 Journal Code: 9107378

121234606 CA: 121(20)234606w JOURNAL
Use of cetylpyridinium chloride in photogalvanic cell for solar energy conversion and storage: thionine-EDTA system
AUTHOR(S): Ameta, Suresh C.; Lodha, Anita; Sahasi, Sapna; Ameta, Rameshwar
LOCATION: Univ. Coll. Sci., Sukhadia Univ., Udaipur, 313 001, India
JOURNAL: Proc. Natl. Acad. Sci., India, Sect. A DATE: 1994 VOLUME: 64 NUMBER: 1 PAGES: 43-8 CODEN: PAIAA3 ISSN: 0369-8203 LANGUAGE: English

11/3,KWIC/26 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

118224926 CA: 118(23)224926z JOURNAL
(1,2-Bis(2-hydroxyphenyl)ethylenediamine)dichloroplatinum(II), a new compound for the therapy of ovarian cancer. III. Detailed evaluation of the antitumor activity of the enantiomeric complexes on the human NIH:OVCAR-3 ovarian cancer cell line
AUTHOR(S): Bernhardt, Gunther; Gust, Ronald; Reile, Herta; Vom Orde, Hans Dieter; Mueller, Richard; Keller, Christoph; Spruss, Thilo; Schoenenberger, Helmut; Burgemeister, Thomas; et al.
LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400
JOURNAL: J. Cancer Res. Clin. Oncol. DATE: 1992 VOLUME: 118 NUMBER: 3 PAGES: 209-15 CODEN: JCROD7 ISSN: 0171-5216 LANGUAGE: English

11/3,KWIC/27 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

117082983 CA: 117(9)82983u JOURNAL
Dichloro-(1-(hydroxyphenyl)-2-phenylethylenediamine)platinum(II) complexes: testing on the human ovarian cancer cell lines NIH: OVCAR 3 and SK OV 3
AUTHOR(S): Bernhardt, Guenther; Mueller, Richard; Gust, Ronald; Reile, Herta; Keller, Christoph; Spruss, Thilo; Schoenenberger, Helmut
LOCATION: Inst. Pharm., Univ. Regensburg, Regensburg, Germany, W-8400
JOURNAL: Arch. Pharm. (Weinheim, Ger.) DATE: 1992 VOLUME: 325 NUMBER: 2 PAGES: 93-9 CODEN: ARPMAS ISSN: 0365-6233 LANGUAGE: English

11/3,KWIC/28 (Item 4 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

113086948 CA: 113(10)86948b JOURNAL
Photogalvanic effect in systems containing basic dyes
AUTHOR(S): Pezza, Leonardo; Neumann, Miguel Guilermo; Gessner, Fergus
LOCATION: Inst. Biocienc., UNESP, 15100, Sao Jose do Rio Preto, Brazil
JOURNAL: Ecletica Quim. DATE: 1989 VOLUME: 14, PAGES: 27-37 CODEN: ECQUDX ISSN: 0100-4670 LANGUAGE: Portuguese

11/3,KWIC/29 (Item 5 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

107069877 CA: 107(8)69877m JOURNAL
The reaction between thionine blue and EDTA as a photochemical pretreatment in determinations of chromium(VI) and peroxydisulfate
AUTHOR(S): Martinez Lozano, C.; Tomas Martinez, V.; Yague, E.
LOCATION: Cent. Coord., CSIC, Spain,
JOURNAL: Afinidad DATE: 1987 VOLUME: 44 NUMBER: 408 PAGES: 115-18 CODEN: AFINAE ISSN: 0001-9704 LANGUAGE: Spanish

Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.

Tags: Comparative Study

Descriptors: *Cartilage, Articular--cytology--CY; *Methacrylates; *Paraffin Embedding; *Phenothiazines--metabolism--ME; Aged; Cartilage, Articular--anatomy and histology--AH; Chondroitin Lyases--metabolism--ME; Decalcification Technique; Glycosaminoglycans--metabolism--ME; Humans; Phenazines; Plastic Embedding; Sensitivity and Specificity; Staining and Labeling--methods--MT

CAS Registry No.: 0 (Glycosaminoglycans); 0 (Methacrylates); 0 (Phenazines); 0 (Phenothiazines); 477-73-6 (safranine T); 581-64-6 (thionine); 868-77-9 (hydroxyethyl methacrylate)

Enzyme No.: EC 4.2.2.- (Chondroitin Lyases)

Record Date Created: 19930412

Record Date Completed: 19930412

12120265 PMID: 9421187

Mechanisms by which thionin **induces susceptibility of S49 cell membranes to extracellular phospholipase A2.**

Wilson H A; Huang W; Waldrip J B; Judd A M; Vernon L P; Bell J D
Department of Zoology, Brigham Young University, Provo, UT 84602, USA.

Biochimica et biophysica acta (NETHERLANDS) Nov 15 1997, 1349 (2) p142-56, ISSN 0006-3002 Journal Code: 0217513
Contract/Grant No.: GM-49710; GM; NIGMS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
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Whereas cells normally resist attack by PLA2, they become susceptible under certain pathological conditions. To ascertain the regulatory mechanisms that induce cellular susceptibility to PLA2, the effect of **thionin** on S49 cells was examined in the presence of PLA2. **Thionin** alone was unable to evoke hydrolysis of the lipid bilayer. Likewise, the addition of PLA2 alone caused production of only a minimal amount of free fatty acid. However, **thionin** and PLA2 together resulted in significant hydrolysis of the cell membrane. **Thionin** caused perturbation of the bilayer structure as suggested by the changes in the emission spectra of laurdan and the permeability of the membrane to propidium iodide. These changes correlated quantitatively with the susceptibility of the lipid bilayer to PLA2. Furthermore, **thionin** induced a modest increase in intracellular Ca2+. The source of this Ca2+ was the extracellular fluid since **EDTA** in the extracellular medium inhibited the Ca2+ influx. Moreover, cobalt chloride, a universal Ca2+ channel blocker, prevented the rise in intracellular Ca2+, the uptake of propidium iodide, and the susceptibility to PLA2 induced by **thionin** . In contrast, the changes in the laurdan emission caused by the **thionin** were not affected by the cobalt. Furthermore, incubation of the cells with the calcium ionophore A23187 also caused the cells to become susceptible to PLA2. We hypothesize that **thionin** causes S49 cell membranes to become susceptible to PLA2 by a Ca2+-dependent perturbation of the bilayer structure.

Tags: Research Support, U.S. Gov't, P.H.S.
Descriptors: *Phenothiazines--pharmacology--PD; *Phospholipases A --pharmacology--PD; Animals; Arachidonic Acid--metabolism--ME; Calcimycin --pharmacology--PD; Calcium--metabolism--ME; Cell Membrane--metabolism--ME; Lipid Bilayers--metabolism--ME; Lymphoma--metabolism--ME; Mice; Tumor Cells, Cultured
CAS Registry No.: 0 (Lipid Bilayers); 0 (Phenothiazines); 506-32-1 (Arachidonic Acid); 52665-69-7 (Calcimycin); 581-64-6 (thionine); 7440-70-2 (Calcium)
Enzyme No.: EC 3.1.1.- (Phospholipases A)
Record Date Created: 19980115
Record Date Completed: 19980115

10106631 PMID: 7680580

Thionin **staining of paraffin and plastic embedded sections of cartilage.**

Bulstra S K; Drukker J; Kuijer R; Buurman W A; van der Linden A J

Department of Orthopaedic Surgery, University Hospital Maastricht, State University of Limburg, The Netherlands.

Biotechnic & histochemistry - official publication of the Biological Stain Commission (UNITED STATES) Jan 1993, 68 (1) p20-8, ISSN 1052-0295 Journal Code: 9107378

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

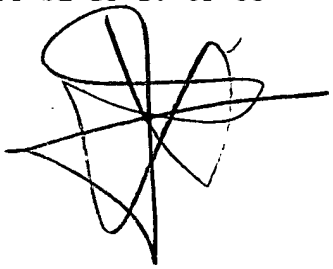
The usefulness of **thionin** for staining cartilage sections embedded in glycol methacrylate (GMA) and the effect of decalcification on cartilage sections embedded in paraffin and GMA were assessed. Short decalcification periods using 5% formic acid or 10% **EDTA** did not influence the staining properties or the morphology of cartilage matrix and chondrocytes. The standard stain safranin O-fast green for differential staining of cartilage was used as control in these experiments. Prolonged exposure of safranin O stained sections to fast green resulted in disappearance of the safranin O stained matrix, thereby hampering the quantitative measurement of negatively charged glycosaminoglycans (GAG). **Thionin** stained evenly throughout all cartilage layers, independent of the staining times. In contrast to safranin O, **thionin** did not show metachromasia in nondehydrated cartilage sections, which made it more suitable for assessing cartilage quality in GMA embedded cartilage. To evaluate the selectivity of **thionin** staining in cartilage, chondroitinase ABC and trypsin digestions were carried out. **Thionin** staining was prevented by these enzymes in the territorial matrix, representing the interlacunar network and the chondrocyte capsule. Staining with **thionin** of the interterritorial matrix was only slightly reduced, possibly representing keratan sulfate and hyaluronic acid in cartilage of elderly patients. Comparison of **thionin** stained GMA embedded cartilage with safranin O stained paraffin embedded sections showed significant similarity in optical densitometry, indicative of the specificity of **thionin** bound to negatively charged GAG in cartilage. In GMA embedded cartilage morphology was relatively intact compared to paraffin embedded sections due to less shrinkage of chondrocytes and the interlacunar network.

Tags: Comparative Study

THIONINE PYRULARIA CONTENANT DES IMMUNOTOXINES AINSI QUE DES CONJUGUES SIMILAIRES AUX IMMUNOTOXINES

Patent Applicant/Assignee:
THERA PRO,
Inventor(s):
VERNON Leo P,
RAEL Eppie D,
GASANOV Sardar E,

Patent and Priority Information (Country, Number, Date):
Patent: WO 9641608 A2 19961227
Application: WO 96US8811 19960605 (PCT/WO US9608811)
Priority Application: US 95479799 19950607
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 12214



Fulltext Availability:
Detailed Description

Detailed Description
... cytoplasmic target and kill the cell

Another immunotoxin which has been developed contains the toxin **purothionin** , isolated from barley flour, conjugated to the monoclonal antibody 225.28S. Unlike the other toxins used to date, **purothionin** does not need to be internalized to be cytotoxic. Instead, **purothionin** binds to the cell membrane where it disrupts the phospholipid bilayer causing cell death. Thus, **purothionin** is never exposed to the proteases found in the cytosol and lysosomes of the host cell

Unfortunately, conjugated **purothionin** is approximately 10,000 times less toxic than the ribosome inactivating toxins (1). As a result, the **purothionin** immunotoxin is only ...the mixture of anti-CD5 and PT in phosphate buffer (pH 6.9, 1 mM **EDTA**) by cation exchange HPLC. SCX 83 C13-ETI Hydropore column was equilibrated with 0.02 M Na2HPO4, pH 6.9, 1 mM **EDTA** . A salt gradient was established with 1 M NaCl and monitored as indicated by broken...the toxin's binding site. This is especially true given the contradictory data for the **purothionin** immunotoxin

5.5.4. Immunotoxin Specificity
The specificity of PT immunotoxin for CD5+ cells was...M of 2-Iminothiolane-HC1 in 0.02 M Na2HPO4 (pH 7.0), 1 mM **EDTA** . Anti-CD5 at 104M was incubated at 37(deg) ...10-4 M of SPDP in 0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . The derivatized proteins were separated from the reactants by dialysis against 0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . Derivatized PT and anti-CD5 were incubated together overnight at room temperature in 0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . Unreacted PT was removed by dialysis (14,000 MW cutoff) against water. The reaction mixture...Company, Inc., Woburn, MA) equilibrated with 0.02 M Na2HPO4 (pH 6.9), 1 mM **EDTA** . A salt gradient was established with buffer containing 1 M NaCl (Figure 4). Fractions...6). Samples for SDS-PAGE were dissolved in buffer (20 mM Tris-HC1, 2 mM **EDTA** , 5% SDS, 0.01% Bromophenol Blue) and applied to an 8-25% acrylamide gradient gel...mM Tris-HC1 buffer (pH 7.5) containing 0.1 M NaCl and 1 mM **EDTA** . 5-doxylstearic acid was used as a spin

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Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**Q9T0P2_WHEAT**
Primary accession number	**Q9T0P2**
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004

Name and origin of the protein

Protein name	**Beta purothionin [Precursor]**
Synonyms	None
Gene name	**Name: Pur-A1**
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
Van Campenhout S., Sagi L., Vander Stappen J., Volckaert G.;
"Characterisation of type-I thionin loci from the A, B, D and R genomes of wheat and rye.";
Theor. Appl. Genet. 96:80-86(1998).

Comments

- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Cross-references

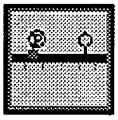
EMBL	X96445; CAA65312.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	A91945; VSWTA1.
HSSP	P01543; 1BHP. [HSSP ENTRY / PDB]
GO	GO:0006952; Biological process: defense response *(inferred from electronic annotation).*
	QuickGo view.
	IPR001010; Thionin.

InterPro	Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
ProtoMap	Q9T0P2.
PRESAGE	Q9T0P2.
ModBase	Q9T0P2.
SMR	Q9T0P2; 90B5C8155730126E.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Plant defense; **Signal**; **Thionin**.

Features

 Feature table viewer

Key	From	To	Length	Description
SIGNAL	1	27	27	*Potential.*
CHAIN	28	72	45	*Potential.*

Sequence information

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14715 Da** [This is the MW of the unprocessed precursor]

CRC64: **90B5C8155730126E** [This is a checksum on the sequence]

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        70         80         90        100        110        120
TSGLSCPKDF PKLVLESNSD EPDTMEYCNL ECRSSLCDYM VNAAADDEEM KLYVEQCGDA

       130
CVNFCNADAG LTSLDA
```

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 ScanProsite, MotifScan

 Search the SWISS-MODEL Repository

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Entry information

Entry name	**Q9T0P2_WHEAT**
Primary accession number	**Q9T0P2**
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 26, March 2004

Name and origin of the protein

Protein name	**Beta purothionin [Precursor]**
Synonyms	None
Gene name	**Name: Pur-A1**
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
Van Campenhout S., Sagi L., Vander Stappen J., Volckaert G.;
"Characterisation of type-I thionin loci from the A, B, D and R genomes of wheat and rye.";
Theor. Appl. Genet. 96:80-86(1998).

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GO	GO:0006952; Biological process: defense response *(inferred from electronic annotation).* QuickGo view. IPR001010; Thionin.

InterPro	Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
ProtoMap	Q9T0P2.
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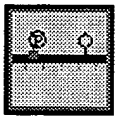
ModBase	Q9T0P2.
SMR	Q9T0P2; 90B5C8155730126E.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Plant defense; **Signal**; **Thionin**.

Features


Feature table viewer

Key	From	To	Length	Description
SIGNAL	1	27	27	*Potential.*
CHAIN	28	72	45	*Potential.*

Sequence information

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14715 Da** [This is the MW of the unprocessed precursor]

CRC64: **90B5C8155730126E** [This is a checksum on the sequence]

```
        10         20         30         40         50         60
MGSKGLKGVM VCLLILGLVL EQVQVEGKSC CKSTLGRNCY NLCRARGAQK LCANVCRCKL

        70         80         90        100        110        120
TSGLSCPKDF PKLVLESNSD EPDTMEYCNL ECRSSLCDYM VNAAADDEEM KLYVEQCGDA

       130
CVNFCNADAG LTSLDA
```

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BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)


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Swiss-Prot family/domain classification: plant thionin (TC 1.C.44) family

Family/domain

plant thionin (TC 1.C.44) family

Hierarchical classification

all families and domains
- family
  - **plant thionin (TC 1.C.44) family**

CC SIMILARITY line

This is the list of all Swiss-Prot entries containing the line:

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CC -!- SIMILARITY: Belongs to the plant thionin (TC 1.C.44) family.
```

extracted from the index of CC SIMILARITY lines.

Swiss-Prot entries

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CRAM_CRAAB  (P01542), THN1_VISAL  (P01537), THN1_WHEAT  (P01544),
THN21_ARATH (Q42596), THN22_ARATH (Q42597), THN23_ARATH (Q8VZK8),
THN24_ARATH (Q9C8D6), THN2_VISAL  (P32880), THN2_WHEAT  (P32032),
THN3_HORVU  (P08772), THN3_VISAL  (P01538), THN5_HORVU  (P09617),
THN5_WHEAT  (Q05806), THN6_HORVU  (P09618), THN7_HORVU  (Q42838),
THNA_HORVU  (P01545), THNA_PHOLI  (P01540), THNB_HORVU  (P21742),
THNB_PHOLI  (P59358), THNB_VISAL  (P08943), THNB_WHEAT  (P01543),
THNC_VISAL  (P83554), THND_HELPU  (P60057), THNX_HORVU  (Q8H0Q5),
THN_BRARP   (Q9SBK8), THN_DENCL   (P01541), THN_PHOTO   (P01539),
THN_PYRPU   (P07504)
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[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THNB_WHEAT**
Primary accession number	**P01543**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 01, July 1986
Sequence was last modified in	Release 36, July 1998
Annotations were last modified in	Release 47, May 2005

Name and origin of the protein

Protein name	**Purothionin A-I [Precursor]**
Synonym	**Beta-purothionin**
Gene name	**Name: THI1.3**
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
STRAIN=cv. Rosella;
Hughes P.A., Llewellyn D.L., Whitecross M.;
Submitted (JUN-1997) to the EMBL/GenBank/DDBJ databases.

[2] PROTEIN SEQUENCE OF 28-72.
STRAIN=cv. Manitoba 3;
MEDLINE=78026451;PubMed=914810 [NCBI, ExPASy, EBI, Israel, Japan]
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast from wheat flour.";
J. Biochem. 82:753-767(1977).

[3] PROTEIN SEQUENCE OF 28-72.
Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;
"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";

Agric. Biol. Chem. 39:2269-2270(1975).
[4] PROTEIN SEQUENCE OF 28-72.
MEDLINE=77046666;PubMed=990986 [NCBI, ExPASy, EBI, Israel, Japan]
Mak A.S., Jones B.L.;
"The amino acid sequence of wheat beta-purothionin.";
Can. J. Biochem. 54:835-842(1976).
[5] X-RAY CRYSTALLOGRAPHY (1.7 ANGSTROMS).
DOI=10.1107/S0907444995002976;PubMed=15299761 [NCBI, ExPASy, EBI, Israel, Japan]
Stec B., Rao U., Teeter M.M.;
"Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: structure of beta-purothionin at 1.7-A resolution.";
Acta Crystallogr. D 51:914-924(1995).

Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

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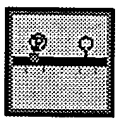
Cross-references

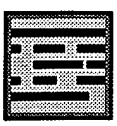
EMBL	AF004018; AAB71137.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PDB	1BHP; X-ray; @=28-71.[ExPASy / RCSB / EBI]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
BLOCKS	P01543.
ProtoNet	P01543.
ProtoMap	P01543.
PRESAGE	P01543.
DIP	P01543.
ModBase	P01543.
SMR	P01543; A855C815519EDA24.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

3D-structure; **Direct protein sequencing**; **Plant defense**; **Plant toxin**; **Signal**; **Thionin**; **Toxin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	27	27	
CHAIN	28	72	45	Purothionin A-I.
CHAIN	73	136	64	Acidic protein.
DISULFID	30	66		
DISULFID	31	58		
DISULFID	39	56		
DISULFID	43	52		
STRAND	29	31	3	
HELIX	34	43	10	
TURN	44	46	3	
HELIX	49	55	7	
TURN	56	57	2	
STRAND	58	60	3	
TURN	68	69	2	

Sequence information

Length: **136 AA** [This is the length of the unprocessed precursor]

Molecular weight: **14625 Da** [This is the MW of the unprocessed precursor]

CRC64: **A855C815519EDA24** [This is a checksum on the sequence]

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MGSKGLKGVM VCLLILGLVL EQVQVEGKSC CKSTLGRNCY NLCRARGAQK LCANVCRCKL

        70         80         90        100        110        120
TSGLSCPKDF PKLVLESNSD EPDTMEYCNL GCRSSLCDYI VNAAADDEEM KLYVEQCGDA

       130
CVNFCNADAG LTSLDA
```

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Report form for errors/updates in this Swiss-Prot entry

BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

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NiceProt View of Swiss-Prot: P01544

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THN1_WHEAT**
Primary accession number	**P01544**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 01, July 1986
Sequence was last modified in	Release 26, July 1993
Annotations were last modified in	Release 47, May 2005

Name and origin of the protein

Protein name	**Alpha-1-purothionin [Precursor] [Fragment]**
Synonym	**Purothionin A-II**
Gene name	**Name: THI1.1** Synonyms: PUR-D1
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
TISSUE=Endosperm;
DOI=10.1104/pp.106.3.1221;MEDLINE=95125120;PubMed=7824649 [NCBI, ExPASy, EBI, Israel, Japan]
Castagnaro A., Marana C., Carbonero P., Garcia-Olmedo F.;
"cDNA cloning and nucleotide sequences of alpha 1 and alpha 2 thionins from hexaploid wheat endosperm.";
Plant Physiol. 106:1221-1222(1994).

[2] PROTEIN SEQUENCE OF 17-61.
STRAIN=cv. Manitoba 3;
MEDLINE=78026451;PubMed=914810 [NCBI, ExPASy, EBI, Israel, Japan]
Ohtani S., Okada T., Yoshizumi H., Kagamiyama H.;
"Complete primary structures of two subunits of purothionin A, a lethal protein for brewer's yeast

from wheat flour.";
J. Biochem. 82:753-767(1977).

[3] PROTEIN SEQUENCE OF 17-61.
Ohtani S., Okada T., Kagamiyama H., Yoshizumi H.;
"The amino acid sequence of purothionin A, a lethal toxic protein to brewer's yeast from wheat.";
Agric. Biol. Chem. 39:2269-2270(1975).

[4] PROTEIN SEQUENCE OF 17-61.
STRAIN=cv. Manitou;
Jones B.L., Mak A.S.;
"Amino acid sequences of the two alpha-purothionins of hexaploid wheat.";
Cereal Chem. 54:511-523(1977).

[5] X-RAY CRYSTALLOGRAPHY (2.5 ANGSTROMS).
MEDLINE=91045879;PubMed=2235992 [NCBI, ExPASy, EBI, Israel, Japan]
Teeter M.M., Ma X.-Q., Rao U., Whitlow M.;
"Crystal structure of a protein-toxin alpha 1-purothionin at 2.5A and a comparison with predicted models.";
Proteins 8:118-132(1990).

Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

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Cross-references

EMBL	X70666; CAA50004.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PDB	2PLH; X-ray; @=17-60.[ExPASy / RCSB / EBI]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
BLOCKS	P01544.
ProtoNet	P01544.
ProtoMap	P01544.
PRESAGE	P01544.
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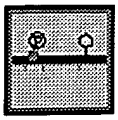
Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
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SIGNAL	<1	16	>16	
CHAIN	17	61	45	Alpha-1-purothionin.
CHAIN	62	126	65	Acidic protein.
DISULFID	19	55		
DISULFID	20	47		
DISULFID	28	45		
DISULFID	32	41		
STRAND	18	20	3	
HELIX	23	32	10	
TURN	33	35	3	
HELIX	38	45	8	
TURN	46	46	1	
STRAND	47	49	3	

Sequence information

Length: **126 AA** [This is the length of the partial sequence of the unprocessed precursor]

Molecular weight: **13526 Da** [This is the MW of the partial sequence of the unprocessed precursor]

CRC64: **FF7310D921C4EE30** [This is a checksum on the sequence]

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        70         80         90        100        110        120
KLALESNSDE PDTIEYCNLG CRSSVCDYMV NAAADDEEMK LYVENCADAC VSFCNGDAGL

PSLDAY
```

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ScanProsite, MotifScan

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Plant Physiol. (1994) 106: 1221-1222

Plant Gene Register

cDNA Cloning and Nucleotide Sequences of α_1 and α_2 Thionins from Hexaploid Wheat Endosperm

Atilio Castagnaro[1], Carmen Maraña[2], Pilar Carbonero, and Francisco García-Olmedo*

Laboratorio de Bioquímica y Biología Molecular, Escuela Tecnica Superior de Ingenieros Agrónomos, Universidad Politecnica de Madrid, E-28040 Madrid, Spain

Thionins are homologous Cys-rich proteins of about 5 kD that have been isolated from different tissues in a wide range of plant taxa and are active against plant pathogens both in vitro and in vivo (Fernández de Caleya et al., 1972; García-Olmedo et al., 1992; Carmona et al., 1993). The available amino acid sequences (either directly determined or deduced from cDNAs) have been classified into five well-defined structural types. Two of these types, I and V, are present in wheat endosperm (Castagnaro et al., 1992; García-Olmedo et al., 1992). Type I corresponds to those thionins included in the original purothionin mixture obtained from wheat flour by Balls and co-workers (Balls et al., 1942). This mixture was resolved through CM-cellulose chromatography into two components, α and β purothionins (Redman and Fisher, 1968). The presence of structural genes for the α purothionin fraction in the long arms of chromosomes 1B and 1D of hexaploid wheat and for the β component in the long arm of chromosome 1A was subsequently demonstrated (Fernández de Caleya et al., 1976). The α purothionin fraction was later resolved by ion-exchange chromatography into two components, α_1 and α_2, whose amino acid sequences differ in six positions (Jones and Mak, 1977) and whose genes are respectively located on chromosomes 1B and 1D (Fernández de Caleya et al., 1976).

Here we report two cDNA clones, pTTH1 and pTTH14, respectively encoding the precursors of α_2 and α_1 purothionins (Table I). The deduced amino acid sequences for the mature protein domain are identical to those directly determined by Jones and Mak (1977). These clones were isolated under nonstringent conditions (58°C) from a cDNA library prepared from developing wheat endosperm, using a barley α-thionin cDNA probe (Ponz et al., 1986). Besides the mature thionin domain, the two precursors contain a signal peptide and a long C-terminal acidic protein, which is in line with the previously observed conservation of precursor structure across types (see García-Olmedo et al., 1992), even across extremely divergent types (Castagnaro et al., 1992), and suggests that all the precursors undergo similar co-translational and posttranslational processing steps (Ponz et al., 1983). Nucleotide sequences encoding the mature protein domain are more divergent (7.4% nucleotide substitutions) than the flanking sequences (signal peptide, 4%; acidic peptide, 3.5%; 3′ noncoding region, 2.5%) and the stop codon is three nucleotides downstream in the α_1 sequence.

Table I. *Characteristics of cDNAs encoding α_1 and α_2 thionins from hexaploid wheat*

Organism:
Triticum aestivum L. cv Chinese Spring.
Loci and Products:
Pur-B1, α_1 thionin; *Pur-D1*, α_2 thionin.
Relevant Feature of the Products:
Activity against plant pathogens both in vitro and in vivo.
Location in Genome:
Long arms of chromosomes 1B (α_1) and 1D (α_2) within a few kb of type-V thionin genes.
Techniques:
cDNA cloning and dideoxynucleotide sequencing of both DNA strands.
Method of Isolation and Subsequent Identification:
Clones isolated from a cDNA library of developing wheat endosperm by hybridization with the insert of barley thionin cDNA and pTH-1. Nucleotide sequences were compared to amino acid sequences of proteins.
Expression Characteristics:
Developmentally regulated, endosperm specific, synchronous with type V genes (8–25 d after pollination).
Features of cDNAs Structure:
Clones encode thionin precursors with three domains (signal peptide, mature protein, and C-terminal acidic protein).
Subcellular Localization:
Periphery of the protein bodies.
Antibodies:
Not available, but antibodies against barley type I thionins cross-react.

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The EMBL accession numbers for the sequences reported in this article are X770660 (pTTH1) and X70665 (pTTH14).

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NiceProt View of Swiss-Prot: P01545

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**THNA_HORVU**
Primary accession number	**P01545**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 01, July 1986
Sequence was last modified in	Release 02, October 1986
Annotations were last modified in	Release 47, May 2005

Name and origin of the protein

Protein name	**Alpha-hordothionin [Precursor]**
Synonym	**Purothionin II**
Gene name	**Name: THI1.1**
From	Hordeum vulgare (Barley) [TaxID: 4513]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Hordeum.

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DOI=10.1016/0378-1119(88)90199-0;MEDLINE=89108011;PubMed=2850969 [NCBI, ExPASy, EBI, Israel, Japan]
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STRAIN=cv. Bomi;
TISSUE=Starchy endosperm;
DOI=10.1002/1522-2683(200011)21:17<3693::AID-ELPS3693>3.0.CO;2-I;MEDLINE=21088911;PubMed=11271488 [NCBI, ExPASy, EBI, Israel, Japan]
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Comments

- ***FUNCTION***: Thionins are small plant proteins which are toxic to animal cells. They seem to exert their toxic effect at the level of the cell membrane. Their precise function is not known.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Copyright

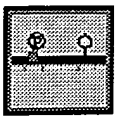
Cross-references

EMBL	X05901; CAA29330.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] M23080; AAA32966.1; ALT_INIT. [EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	JA0087; VSBH2.
HSSP	P01544; 2PLH. [HSSP ENTRY / PDB]
InterPro	IPR001010; Thionin. Graphical view of domain structure.
Pfam	PF00321; Thionin; 1. Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
BLOCKS	P01545.
ProtoNet	P01545.
ProtoMap	P01545.
PRESAGE	P01545.
DIP	P01545.
ModBase	P01545.
SMR	P01545; 70C1BD787A9D1C46.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Direct protein sequencing; **Multigene family**; **Plant defense**; **Plant toxin**; **Signal**; **Thionin**; **Toxin**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	18	18	
CHAIN	19	63	45	Alpha-hordothionin.
CHAIN	64	127	64	Acidic protein.
DISULFID	21	57		
DISULFID	22	49		
DISULFID	30	47		
DISULFID	34	43		

Sequence information

Length: **127 AA** [This is the length of the unprocessed precursor]

Molecular weight: **13597 Da** [This is the MW of the unprocessed precursor]

CRC64: **70C1BD787A9D1C46** [This is a checksum on the sequence]

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        10         20         30         40         50         60
MVCLLILGLV LEQVQVEGKS CCRSTLGRNC YNLCRVRGAQ KLCAGVCRCK LTSSGKCPTG

        70         80         90        100        110        120
FPKLALVSNS DEPDTVKYCN LGCRASMCDY MVNAAADDEE MKLYLENCGD ACVNFCNGDA

GLTSLTA
```

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THE JOURNAL OF BIOCHEMISTRY

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ARTICLES

Amino acid sequence of a purothionin homolog from barley flour

Y. Ozaki, K. Wada, T. Hase, H. Matsubara, T. Nakanishi and H. Yoshizumi

A purothionin homolog was isolated from barley flour and purified by CM-52 column chromatography. It showed potent lethal activity towards brewer's yeast and its complete amino acid sequence was determined to be as follows. Lys-Ser-Cys-Cys-Arg-Ser-Thr-Leu-Gly-Arg-Asn-Cys-Tyr-Asn-Leu-Cys-Arg-Val-Ar g-Gly-Ala-Gln-Lys-Leu-Cys-Ala-Gly-Val-Cys-Arg-Cys-Lys-Leu-Thr-Ser-Ser-Gly- Lys-Cys-Pro-Thr-Gly-Phe-Pro-Lys. It thus consists of 45 amino acid residues with 8 cysteines. The number of amino acid residues and the positions of the 8 cysteines are identical with those of wheat purothionins. There is a high degree of homology in the primary structures of these proteins.

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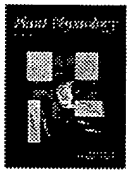

Plant Physiology ▸HOME

F. T. Lay, F. Brugliera, and M. A. Anderson
Isolation and Properties of Floral Defensins from Ornamental Tobacco and Petunia
Plant Physiology, March 1, 2003; 131(3): 1283 - 1293.
[Abstract] [Full Text] [PDF]

Acta Cryst. (1995). D51, 914–924

Refinement of Purothionins Reveals Solute Particles Important for Lattice Formation and Toxicity. Part 2: Structure of β-Purothionin at 1.7 Å Resolution

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(*Received* 29 *June* 1994; *accepted* 1 *March* 1995)

Abstract

The crystal structure of β-purothionin (β-PT) has been determined at 1.7 Å resolution. β-PT and previously solved α_1-PT belong to a family of membrane-active plant toxins homologous to crambin. β-PT crystallizes in the same space group as α_1-PT (*I*422) but with the *c* axis 3 Å longer than α_1-PT. The unit-cell dimensions of β-PT crystals are $a = b = 53.94$ and $c = 72.75$ Å. Two data sets were collected on a multiwire area detector, each with R_{sym} around 6.0%, and were merged to get a single data set at 1.7 Å ($R_{merge} = 9.6\%$). The X-ray structure of α_1-PT was used to build a starting model for β-PT. The β-PT model was refined using the program *PROLSQ* from 10 to 1.7 Å resolution to an *R* factor of 19.8% with very good geometry. The final structure contains 439 atoms including 337 protein atoms, 77 waters, two acetates, two glycerols and one phosphate. The high-resolution structure of the β-PT agreed well with that of the lower resolution α_1-PT structure only after the latter was extensively rerefined. Both refinements revealed phosphate and glycerol molecules which are important in lattice formation. The binding of phosphate and glycerol molecules to purothionins (PT) was confirmed by NMR and was implicated in the biological activity of toxins. Modeling of phospholipid binding to PT based on glycerol and phosphate-binding site could shed light on the lytic toxicity of this protein-toxin family. Although the structures of α_1-PT and β-PT preserve the overall fold of crambin, they exhibit key differences that are directly relevant to the toxicity of thionins.

1. Introduction

Thionins constitute an important family of plant toxins (Bohlman & Apel, 1991) which are ~60% homologous to crambin. The crystal structure of a member of this family, α_1-purothionin (α_1-PT), has recently been determined in our laboratory at 2.5 Å resolution (Teeter, Ma, Rao & Whitlow, 1990; Rao, Stec & Teeter, 1995). Because of inherent problems with the α_1-PT crystals, however, higher resolution data could not be collected from these crystals. In order to better understand the toxic behavior of these thionins (Carrasco *et al.*, 1981), we crystallized β-purothionin (β-PT) and obtained well ordered crystals. After several modifications to initial crystallization conditions, crystals diffracting to 1.7 Å were obtained.

β-PT has about 88% sequence identity with α_1-PT. It differs from α_1-PT only at five residues out of 45 residues. The important changes are at positions 27 and 42 where glycine residues are replaced by asparagine and aspartic acid, respectively (Fig. 1). These changes should not induce major changes in the backbone structure. The structure of β-PT should, therefore, be very similar to that of α_1-PT. With this knowledge, a crystallographic study of β-PT was undertaken in order to (i) ascertain the structural similarities between the two purothionins and (ii) determine the structure at higher resolution so as to understand the toxic behavior in greater detail. We used homology modeling to build a model of β-PT by replacing those residues of the α_1-PT model which are different in β-PT. The resulting model has been used to solve the X-ray structure.

This paper describes the crystallization of β-PT, its crystal structure determination at 1.7 Å resolution and its comparison to the α_1-PT structure. The sequence differences between crambin and these thionins contribute directly to the membrane activity of the toxins.

2. Experimental procedures

2.1. *Crystallization of β-PT*

2.1.1. *Crystallization conditions.* Plate-like crystals of β-PT were grown by sitting-drop vapor-diffusion methods similar to those used for growing crystals of α_1-PT (Teeter *et al.*, 1990). As precipitant we used either 2-methyl-2,4-pentanediol (MPD) or *sec*-butanol. A typical crystallization with *sec*-butanol consisted of a 20 ml dip with 50 mg ml^{-1} β-PT in sodium cacodylate (CAC) buffer at pH 5.9 to which 8 to 15%(*v*/*v*) *sec*-butanol was added. The dip was equilibrated against 12% *sec*-butanol and 17% $MgCl_2$ in 90 m*M* CAC buffer (pH = 5.9) at room temperature. Plate-like crystals were readily obtained in a week.

In the case of MPD as precipitant, crystals were obtained when dips containing 10 or 15% MPD were equilibrated against 10% MPD and 22% $MgCl_2$ in the reservoir. Slightly thicker crystals were obtained when the dips contained a trace amount (1.0%) of dioxane. Most of the crystals had dimensions of 0.2 × 0.2

* To whom correspondce should be addressed.

× 0.05 mm. We also tried different buffers, and crystals were obtained in *N*-(2-acetamido)-iminoacetic acid (ADA) at pH 6. They were more fragile than crystals obtained from CAC.

The presence of $MgCl_2$ in the reservoir seemed critical in all these set ups. Since *sec*-butanol had a phase separation at 20% concentration, higher concentrations could not be used. Hence, $MgCl_2$ was necessary to drive water out of the dip. The crystals obtained by these methods, however, had an intrinsic tendency for twinning: they looked like thin plates stacked one over the other with a slight rotation between layers.

2.1.2. *Stabilization of the crystals.* Initial attempts were made to collect X-ray diffraction data from the plate-like crystals grown from CAC buffer but several problems were encountered. Crystals were very tiny and dissolved quickly when the crystallization boxes were opened. Since they were grown from alcohol at a very critical equilibrium concentration, they redissolved in the mother liquor even at minimal evaporation of the alcohol. Additional problems were posed by the small amount of mother liquor around the crystals. Various methods of crystal mounting such as oil mounting, glovebox mounting, *etc.* were tried to circumvent these problems in vain. Hence we developed a stabilization procedure for the crystals.

Various concentrations of MPD and $(NH_4)_2SO_4$ (AS) were tried both individually and in combination. 100% of ammonium sulfate (AS) alone caused the crystals to dissolve and reappear as precipitate whereas MPD alone resulted in the development of cracks in the crystals. Finally a mixture of 30% saturated AS and 8% MPD seemed to stabilize the crystals well.

2.1.3. *Improvement of the crystal quality.* Because of the role of AS in stabilizing the crystals, attempts were made to crystallize in the presence of AS as well. Large crystals were grown from 20 ml dip containing 15 mg ml^{-1} β-PT, 2% dioxane, 5% MPD and 58% AS in 75 m*M* CAC buffer (pH = 5.9) equilibrated against 10% MPD and 20% $MgCl_2$ in 90 m*M* CAC buffer. The presence of AS prevented drying of the dips by lowering the solubility of the protein.

A thick large crystal was therefore cut to a size of 0.4 × 0.4 × 0.2 mm, stabilized with the previously described solution, and mounted in a glass capillary. The crystal diffracted well (up to 2.1 Å). The β-PT crystals belong to the same space group (*I*422) as α_1-PT with similar cell parameters: $a = b = 53.94$, $c = 72.75$ Å and $\alpha = \beta = \gamma = 90°$. The c dimension of β-PT was larger than for α-PT by ~3 Å.

Eventually, even better crystals of ~0.5 × 0.3 × 0.2 mm size were grown in about 10 d when a mixture of MPD, polyethylene glycol (PEG) and AS was used as precipitant. Here a 20 ml dip containing 50 mg ml^{-1} of β-PT in 90 m*M* CAC with 10% MPD, 2.5% PEG 4000 and 8% AS was equilibrated against a reservoir containing 10% MPD and 20% $MgCl_2$ in 75 m*M* CAC (pH = 5.9). This batch of crystals diffracted to 1.7 Å resolution.

2.2. *Data collection and analysis*

Two different data sets were collected using a Xuong–Hamlin multiwire area detector powered by a Rigaku RU200 rotating-anode generator at 50 kV and 100 mA equipped with a graphite monochromator. A monochromatized Cu $K\alpha$ X-ray beam was used.

The crystal to detector distance was 400 mm, which along with the detector 2θ value (19°) allowed the data collection to 2.1 Å resolution. Intensity and σ of each observation along with all indexing and background corrections were calculated simultaneously with the scanning process.

In this manner, 11 407 observations of 2646 independent reflections were collected for β-PT. The data was reduced by software provided by San Diego Multiwire Systems, the intensities were next reduced by applying the usual Lorentz and polarization corrections, scaled together in 55 increments of ω, and averaged. Final R_{sym} ($R_{sym} = \Sigma_i|\langle I\rangle - I_i|/\Sigma\langle I\rangle$) for the data was 5.63%. This data set was 99% complete to 2.31 Å and contains 66% data to 2.25 Å. The overall fraction of data measured above 2σ is 97%.

A second native data set has been collected on a fresh crystal grown in the presence of mineral oil. Mineral oil, which was a remnant from our previous oil mounting attempts, was present in the droplets and the crystals formed readily on the interface between oil and mother liquor. The better crystal quality and the use of crystal-to-chamber distance of 400 mm with the 2θ value of the detector being −32° allowed the data to be collected up to 1.7 Å resolution. A total of 44 767 reflections comprising of 4595 unique reflections were measured and reduced as described above with R_{sym} being 6.1%. However, due to interruption in the data-collection procedure, we collected only 80% of the data. Therefore, we merged the

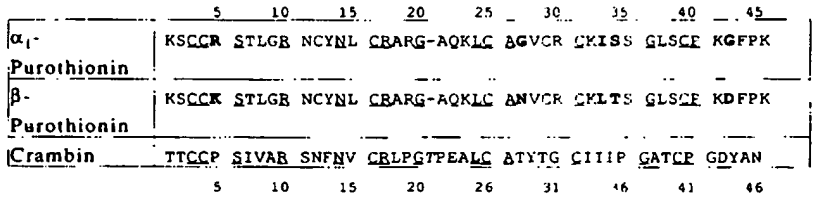

Fig. 1. Sequence alignment of crambin, α_1- and β-purothionin. Substitutions between the forms are in bold and amino acids identical for the family are underlined. Insertion in crambin is in italics.

Table 1. *Summary of various stages of refinement of the β-PT structure*

Stage	Adjustments to the model	Resolution (Å)	Total No. atoms	Total No. waters	No. of others*	No. of cycles	R factor (%)
1	α_1-PT model (R = 18.4) with side chains changed to fit β-PT	2.4	337	0	0	26	26.3
2	Side chains of the residues 5, 27, and 42 were corrected	2.4	419	22	1 MPD 1 ACT	35	21.7
3	Side chains of the residues 1, 19, 30, 34 and 38 were corrected, MPD changed to GLC and POS introduced	2.25	439	439	2 GLC 1 ACT 1 POS	35	18.7
4	Side chains of the residues 23 and 32 and GLC were corrected	1.7	464	72	2 GLC 1 ACT 1 POS	30	22.4
5	Another ACT was introduced and GLC corrected	1.7	483	77	2 GLC 2 ACT 1 POS	48	19.8

* Abbreviations used here: MPD, 2-methyl-2,4-pentanediol; GLC, glycerol; ACT, acetate ion; POS, phosphate ion.

two data sets. They were scaled in different resolution ranges and the data from the second set in the 2.3–1.7 Å resolution range were merged with the 2.3 Å data of the first set. The merged data set contained 5092 reflections with an R_{merge} of 9.6%.

2.3. *Modeling and refinement of the βPT structure*

The model for β-PT was constructed from partially rerefined α_1-PT model at 18.4% R value (reduced from 21.6%, Teeter *et al.*, 1990) by changing the appropriate residues in *FRODO*. All these changed residues were subjected to about 30 cycles of Hermans' geometric refinement (1974) using the *FRODO* package. Because of the small number of changes, global energy minimization was not used. However, side chains were placed in their most statistically favored rotamer positions (Ponder & Richards, 1987).

The resulting model with 337 atoms was used as the starting model for the restrained least-squares refinement. All the refinement was carried out using the *PROLSQS* software package of Hendrickson modified by Sheriff (1987) to include van der Waals restraints on symmetry relations. One cycle of refinement required approximately 25 min of central processing unit (c.p.u.) time on a Vaxstation 3500.

The program *PROTINS* was used to impose intermolecular non-bonded contacts. Restraints on distance, non-bonded contacts, chiral volume and planarity were imposed on additional solute molecules such as glycerol, phosphate and acetate during the refinement. Non-bonded restraints were imposed on the water molecules.

Table 1 summarizes the course of the refinement and Table 2 gives the final refinement parameters. Each round of refinement consisted of a session of model building followed by refinement to convergence. Five rounds of refinement were performed. Typical remodeling included side-chain conformation modification and modeling of new solvent sites. Refinement began with the data above 2σ up to 2.4 Å. After the second round, all the data (above 2σ) to 2.25 Å were added.

Table 2. *Summary of restrained least-squares refinement parameters for β-PT*

	Target σ	R.m.s. deviation
Average ΔF		259.22
R factor*		0.198
R_{error}†		0.072
No. of structure factors ($> 2.5\sigma$)		4966
Structure-factor weight‡	220	343.5
R.m.s. deviations from ideal (Å)		
Bond distances	0.02	0.017
Angle distances	0.03	0.031
Plane 1–4 distances	0.04	0.040
R.m.s. deviation from planarity (Å)	0.015	0.008
R.m.s. deviation from ideal chirality (Å^3)	0.15	0.174
R.m.s. deviations from permitted contact distances (Å)		
Single-torsion contacts	0.5	0.169
Multiple-torsion contacts	0.5	0.193
Possible hydrogen bond	0.5	0.207
R.m.s. deviations from ideal torsion angles (°)		
For planar group (0 or 180)	5.0	2.0
For staggered group (60) or 180)	18.0	19.6
For orthogonal group (90)	20.0	1.8
R.m.s. deviations of the isotropic thermal factor differences (Å^2)		
For main-chain bond	2.0	1.45
For main-chain angle	3.0	1.57

* $R = \sum_h ||F_o||/\sum_h |F_o|$, where h is over all h, k, l.
† $R_{error} = \sum_h |\sigma(F_o)|/\sum_h |F_o|$.
‡ The weight chosen for the structure-factor refinement, the 'target σ' of ΔF, was modeled by the function, $\omega = (1/\sigma)^2$ with $\sigma = 220 + [(-1000.0)(\sin\theta/\lambda - 1/6)]$.

The $F_o - F_c$ map produced after the first 26 cycles of refinement (round 1) showed clearly new conformations for residues 27 and 42 which are different from those of α_1-PT. Asp42 could be fit into the cleft formed by the residues Phe43 and Pro40 in a similar conformation as in crambin. Appropriate changes to these residues along with a minor correction to Lys5 were made during round 2. The side chains of residues such as Arg19, Lys1, Lys32, Lys23 and Lys41 attained new conformations which may be ascribed to the difference in contacts between α_1-PT and β-PT molecules in the crystals. These side chains were modeled during rounds 3 and 4.

2.4. *Solvent/solute modeling*

Criteria used to model the solvent structure evolved during the course of refinement. The initial water model of β-PT was predicted using a template for water around polar groups in proteins derived from the analysis of crystal structures at 1.4 Å or better resolution (Roe & Teeter, 1993). This method can be useful for an initial crystallographic refinement of water as well as for model building in general. Templates for water prediction had been derived from high-resolution structures by superimposing the hydrogen-bonding groups around Arg, Glu, Asp, Asn and Gln. The waters around these residues were added to the structure that had been refined to convergence without water. The *R*-factor dropped by 2% when these water positions were refined and all but two out of 22 waters added were found to be correct. Subsequent water positions were modeled by inspecting the $F_o - F_c$ electron density and if a peak fell within 2.5 to 3.5 Å of an atom of the model that was capable of hydrogen bonding, the peak was accepted as a site for solvent. Only the first shell of the solvent was modeled in the beginning.

As in the case of α_1-PT, large peaks of difference densities were observed on the polar face of the protein. An acetate ion was modeled at the intersection of two twofold axes. In the density close to Tyr13, an MPD* molecule was introduced at first. MPD was used in crystallization and was thought to have the correct shape and number of atoms to fit the density. Unfortunately, after 25 cycles of refinement the tetrahedral group of disubstituted carbon of the MPD molecule was flattened and distorted, and showed imperfect fit to the density. After careful inspection of the densities and possible head groups of phospholipids, a glycerol molecule was modeled at this site and it refined very well (Fig. 2). The electron-density maps with improved phases clearly showed a new conformation for the glycerol as compared with α_1-PT. The O2 atom bound in α_1-PT to the Gln22 formed a hydrogen bond to the NZ of symmetry-related Lys45 in β-PT. The discussion of the possible sources of glycerol was presented in the previous paper (Rao, Stec & Teeter, 1995).

The two positions at which we had detected the *sec*-butanol in α_1-PT were carefully inspected. The density at the hydrophobic face which corresponded to *sec*-butanol in α_1-PT was larger. Thus, we modeled a second glycerol molecule at this site. Since our crystallization solution did not contain *sec*-butanol, the other *sec*-butanol position, close to the N terminus, proved not to be occupied.

The modeling of a phosphate ion was also not entirely straightforward. The shape of the electron density at the location that corresponded to a phosphate ion in α_1-PT was not indicative of a phosphate ion at the beginning. However at stage 3, a phosphate ion was modeled at this site. Introduction of phosphate along with 35 cycles of refinement at round 3 resulted in a final *R* factor of 17.8% at 2.25 Å. Elevated temperature factors for the phosphate ion and weaker electron density as compared to α_1-PT suggests that it is possible that phosphate was partially replaced by sulfate. After addition of all the data to 1.7 Å resolution at stage 4 and 5 and two modeling sessions, the *R* factor refined to the level of 19.8%. At stage 4 side chains of Lys23 and Lys32 were corrected as indicated by the density at 1.7 Å. At stage 5 an additional acetate molecule was added at the charged cluster near Arg30. The final model has a total of 439 atoms including 337 protein atoms, 77 waters, two glycerol molecules, two acetates and one phosphate.

3. Results and discussion

3.1. *Quality of the structure*

The quality of the refined structure can be assessed using various analyses. The *R*—free method (Brünger,

* Solute molecules were constructed in *QUANTA* (Polygen Corporation, 1991).

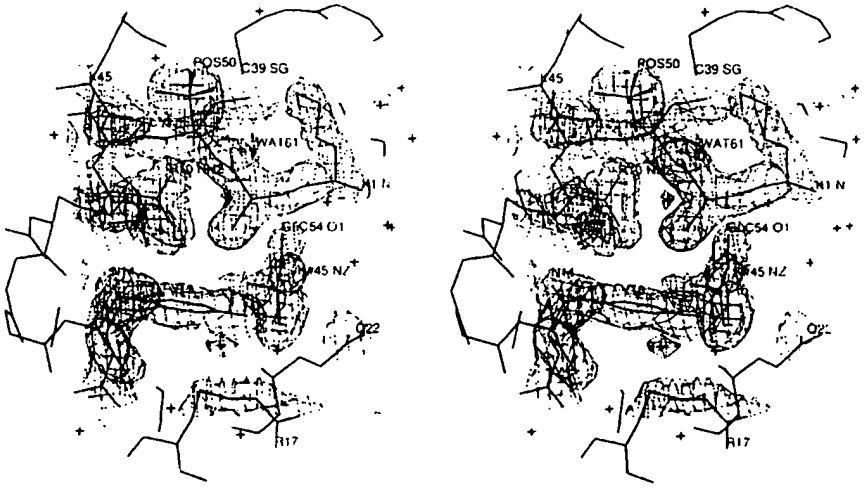

Fig. 2. Stereoview of the of the phosphate–glycerol-binding site in β-PT. Please note conformational changes of Lys1 and the glycerol molecule. The map is contoured at the 1.6σ level.

1992*a*,*b*) showed the decorrelation (difference between the main and the test data sets) in an acceptable range (~10%) with R_{Main} = 19.96% and R_{free} = 28.13%. A Luzzati plot (Luzzati, 1952) shown in Fig. 3 gives a nearly ideal fit of 0.22 Å for positional errors in atomic coordinates.

The r.m.s. deviation in distances from ideal values falls close to or within targeted variances (see Table 2). Only ~5% of all distances in the three categories tabulated in *PROLSQ* fall two standard deviations outside of their ideal values.

The r.m.s. deviation of atoms of planar groups from their fitted planes is 0.008 Å, which is well within the targeted value of 0.015 Å. No planar deviation is beyond two standard deviations of the target variance. The low variance in planar groups reflects, to a large extent, the tight distribution of the ω torsion angle of about 180°. Since a wider ω-angle distribution is found in highly refined structures like crambin (Teeter, Roe, & Heo, 1993) as well as in polypeptides (Ashida, Tsungoae, Tanaka & Yamane, 1991), the restraints were relaxed to 5.0 Å.

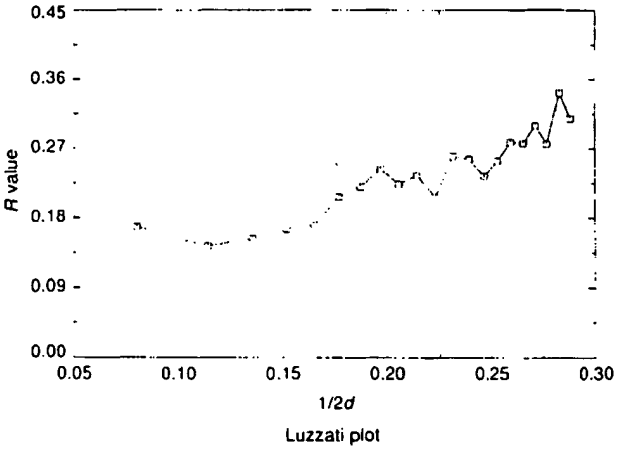

Fig. 3. Luzzati (1952) plot of R factor as a function of resolution ($\sin\theta/\lambda$). The broken lines (Δr = 0.2, 0.25, 0.30) show the theoretical variation in R for non-centric data (10–1.7 Å), when only coordinate errors of the model contribute to the difference between the observed and calculated quantities.

The dihedral angles of the main chain as well as the side chains generally conform well to their expected values. All of the side-chain conformations (χ_1,χ_2) are in allowed regions of Ponder & Richards' library (1987) except four arginines, Arg10, Arg17, Arg19 and Arg30. This deviation of arginines can be explained in terms of crystal contacts or similarities to crambin.

In general, the electron density for the model is excellent (Fig. 4). All of the backbone atoms have uniquely defined positions by the density. Even for the long side chains such as those of arginines, all the atoms, especially atoms in guanidinium groups are clearly visible. Only Arg30 and Lys41 seem to have weaker densities.

Weak negative difference electron density was observed on Asn27 and Asp42. This fact plus relatively high temperature factors for those side chains suggests that these crystals may be a mixed form of α_1- and β-purothionins in the β-lattice. Only those two residues are distinct enough to show the differences in sequence between the two forms. An attempt was made to establish the ratio by refining occupancy of those residues. The final occupancy was ~80% for β-PT

The temperature factor *versus* residue profile (Fig. 5) shows the same type of mobility in both structures and is reminiscent of that for crambin. The temperature factors of β-PT are slightly higher than those of α_1-PT (Fig. 5) as well as those of additional solute molecules. This may be attributed both to the fact that β-PT crystals appear to be a mixture of two forms (see above) and to the looser packing for helix 2 in β-PT lattice. Variation in crystal contacts from crystal to crystal can also explain a relatively high R_{merge} between both data sets.

3.2. *Description of the structure*

The secondary structure of β-PT and overall folding is the same as that of α_1-PT (Teeter *et al.*, 1990; Rao *et al.*, 1995). The α_1- and β-PT structures resemble that of crambin, with the general shape of the Greek capital letter gamma (Γ). The vertical stem (Fig. 6) is comprised of two antiparallel α-helices, and the horizontal arm has a coil in extended conformation and a short

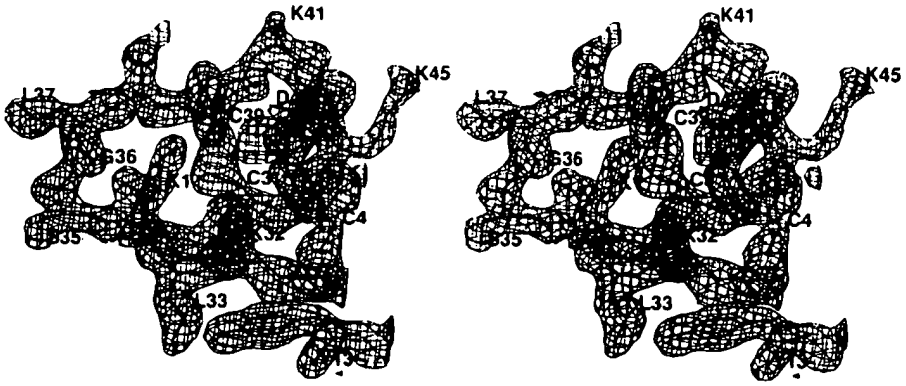

Fig. 4. Stereoscopic view of a typical electron-density map superimposed on the appropriate part of the β-PT model. Residues which make the β-sheet (1–5 and 30–34) are shown. Parts of residues 6, 10, and 43 are also included. The map is contoured at the 1.6σ level.

Table 3. *Intramolecular hydrogen bonds in α_1- and β-PT*

Acceptor	Donor	Distance in β-PT (Å)	Distance in α_1-PT (Å)
Lys23 O	Asn27 OD1	2.92	Not present
Asp42 OD1	Asp42 N	2.85	Not present
Thr34 OG1	Gly36 N	3.04	Not present
Thr34 OG1	Ser35 N	2.98	Not present
Ser34 OG	Ser38 O	Not present	2.91
Thr7 OG1	Thr7 N	2.83	3.12
Thr7 OG1	Leu8 N	2.92	2.84
Ser6 OG	Gly9 N	3.24	2.91
Lys45 OT*	Arg10 NE	2.87	2.62
Lys45 O*	Arg10 NH2	2.92	2.73
Ser2 O*	Arg10 NH2	2.96	2.76
Ser2 OG*	Arg10 NH1	2.92	2.87
Gly20 O	Arg17 NH1	2.81	2.78

* Major 2–10–45 interactions.

antiparallel β-sheet. The residues 2–10–45 which form a tight hydrogen-bonded cluster responsible for holding the stem and the arm together are conserved. In variance to crambin which has three disulfide bonds the toxin structures are crosslinked by four disulfide bridges. The comparison of intramolecular side-chain contacts in both toxins is presented in Table 3.

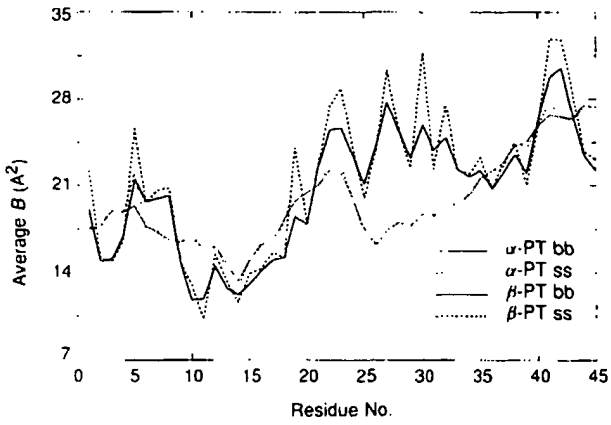

Fig. 5. The comparison of the temperature-factor profiles for α_1-PT and β-PT (heavy dashed and heavy continous lines). Backbone atoms are denoted by bb (in continous lines) while ss denotes side chains (in dashed lines).

Table 4. *Alignment of primary and secondary structures of β-PT with average backbone hydrogen bonds*

Name of secondary-structural element*	Residue span of element	Average φ/ψ (σ φ/ψ) (°)	Average hydrogen-bond distance (Å)
S1	1–4	−114.7(18.6)/ 135.5(15.2)	2.79
T1	5–6	Turn	None
H1	7–18	−63.4(4.5) −39.2(11.7)	2.80
T2	19–21	Helix linker	None
H2	22–28	−69.4(15.8)/ −43.7(9.8)	2.93
T3	29–30	Turn	2.69
S2	31–34	−118.9(15.0)/ 151.2(18.7)	2.79
T4	35–37	β-sheet linker	None
S3	38–39	−110.0/147.0	None
T5	40–44	Type I β-turn	3.21
S4	45	−103.0/172.0	2.75

* Notations used in naming the secondary-structural elements: *H* = α-helix; *S* = β-strand; *T* = reverse turn.

Since the present structure is of higher resolution than that of α_1-PT, an attempt is made to classify different secondary-structural units including the different types of turns. As shown in Table 4 and Fig. 7, the globular fold of β-PT consists of an antiparallel pair of helices (residues 7–18 and 22–28) and two stranded β-sheet (residues 1–4, 31–34 and 45), an extended coil region (residues 38–39) and five turns (residues 5–6, 19–21, 29–30, 35–37 and 40–44). The assignment and average distances of hydrogen bonds formed in different structural units are listed in Table 4. For comparison with crambin refer to Table 4 in Teeter *et al.* (1993).

3.2.1. *Helices.* Two α-helices are found in β-PT (Fig. 7*a*), one with three turns (termed helix *H*1, residues 7–18) and the other with two turns (termed helix *H*2, residues 22–28). The mean torsion angles (φ,ψ) of helices *H*1 and *H*2 are (–63.4, –39.2°) and (–69.4, –43.6°), respectively. Those dihedrals are slightly different from those seen in crambin (–62.1, –39.9°) and (–69.6, –32.0°) indicating a different environment for

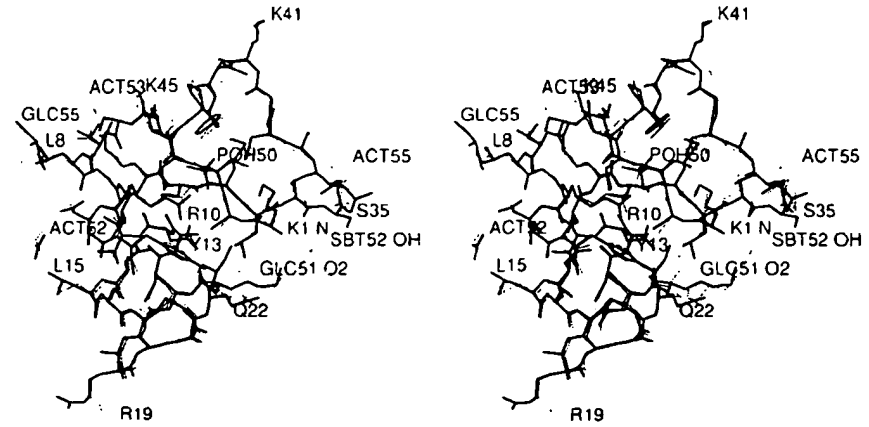

Fig. 6. Superposition of the atomic models of α_1-PT and β-PT showing the difference in conformations of a few of the side chains. α_1-PT is in thin lines and β-PT is in thick lines.

the helices. The *H*1 helix ends with a turn of 3_{10}-helix (Leu15–Ala18).

The backbone hydrogen-bond (O···N) lengths in the helices vary from 2.71 to 3.37 Å with a mean value of 2.94 Å which is close to the expected O—N distances. Asparagine residues are known to introduce distortions to the helices. Although Asn14 did not introduce any major distortions, two other aspargines, 11 and 27, are responsible for small perturbations in the helices. The side-chain N atoms of these aspargines tend to have close encounters with the carbonyl groups of the n_{i+4} residues (Table 2).

It was thought that in crambin Pro19 is responsible for helix termination and the formation of a 3_{10} turn

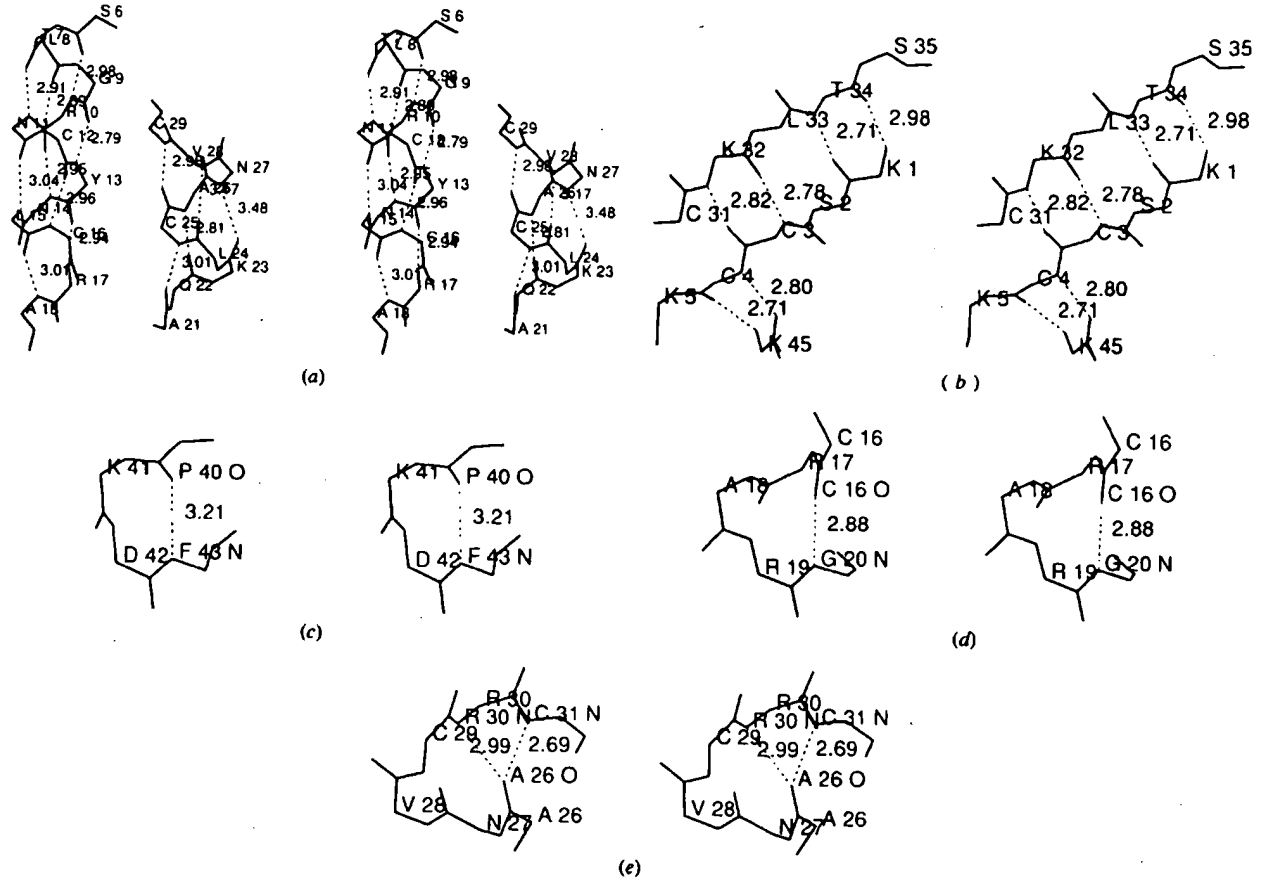

Fig. 7. Backbone atomic models of different secondary structural units in a β-PT. Cα atoms are labeled and hydrogen bonds are shown in broken lines. (*a*) Helices *H*1 (residues 7–18) and *H*2 (residues 21–29). (*b*) β-sheet formed by strands *S*1 (residues 1–4), *S*2 (residues 32–34) and *S*4 (residue 45). (*c*) Turn *T*5 (residues 40–43) in type I β-turn conformation. (*d*) Turn *T*2 between the helices *H*1 and *H*2 showing the α-type twist. (*e*) π-type turn at the C terminus of the helix *H*2 showing $n_i \rightarrow n_{i+5}$ hydrogen bonding between residues 26 and 31.

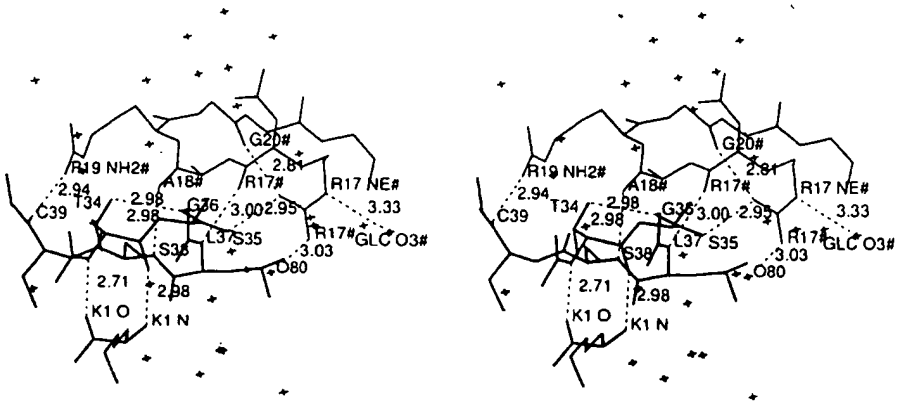

Fig. 8. Stereoview of intermolecular contacts between the symmetry-related molecules involved in interaction *B*. Two backbone hydrogen bonds form a pseudo β-sheet between the end of the *T*4 turn and the *T*2 turn.

towards the end of the helix *H*1. But in both β- and α_1-PT this 3_{10} turn is observed despite the absence of proline at position 19.

3.2.2. *β-strands*. There are four short strands in the structure each containing one to four residues (residues 1–4, 32–34, 38–39 and 46 for the strands *S*1, *S*2, *S*3 and *S*4 respectively). Three of them are hydrogen bonded and form an antiparallel β-sheet (*S*1–*S*2, *S*2–*S*4; Fig. 7*b*) with hydrogen bonds listed in Table 3. The *S*3 strand is not involved in intramolecular sheet formation but is involved in intermolecular contacts (Fig. 8). The average (φ,ψ) torsion angles over all β-strand residues are (−116.5, 145.7°).

3.2.3. *Turns*. There are five turns in the structure of β-PT (residues 5–6, 19–21, 30–31, 35–37, 40–44 for turns *T*1 to *T*5). Turns *T*4 and *T*5 are similar to those of crambin. *T*5 is a classical type I β-turn (see Fig. 7*c*). It has a weak hydrogen bond between the carbonyl group of Pro40 and amide N atom of Phe43 (3.12 Å).

The *T*2 turn between the *H*1 and *H*2 helices is considerably different from that in crambin but very similar to that in α_1-PT. In crambin, due to the presence of Pro at position 19, this turn is a classical type I β-turn. But in β-PT, the deletion of one residue together with the residue change from Pro19 to Arg19 is responsible for the change in conformation of this turn to an α-helical turn ($n_i \rightarrow n_{i+4}$ hydrogen bonding) with the carbonyl O atom of Cys16 bonding to the amide N atom of Gly20 (Fig. 7*d*). The *T*3 turn constitutes another example of a turn architecture where $n_i \rightarrow n_{i+5}$ residues form a hydrogen bond which is reminiscent of the π helix bonding pattern.

3.3. *Crystal packing*

The essential features of the overall crystal packing have been discussed in the previous publication (Teeter *et al.*, 1990) but are repeated here because of key solute reported here mediating these interactions. In brief, there are four kinds of intermolecular interactions, termed *A*–*D*, which help the molecules associate into clusters (Fig. 9*a*). Interaction *B*, because of its hydrophobic nature, forms a hydrophobic dimer whereas interaction *D* forms a polar dimer. Both together result in a tetrameric cluster.

Because of the finding of various solutes in between the interacting surfaces, additional details have been revealed for the polar dimer interaction *D*. More solute-mediated contacts have been found which appear critical not only for the stability of the lattice but also for the lytic activity of the molecules.

3.3.1. *Dimer association*. Two types of dimers can be characterized by looking at the tight contacts at the interaction sites *B* and *D*. The contact *B* could be called a hydrophobic dimer as it is mediated by leucine-ladder type interactions between the first helices of two molecules. The second interaction *D* can be called hydrophilic (it is primarily polar but involves some van der Waals contacts).

Interaction *D* is the strong polar dimer contact between the residues of the first helices of a molecule with the corresponding residues of the twofold symmetry-related molecule. As seen in Table 5, the protein–protein interaction in this dimer is both through intermolecular hydrogen bonds between two aspargines, Asn11 and Asn14 and through van der Waals contact between the CG atoms of symmetry-related Arg10 and CG of Leu15. But a number of other polar and charged residues such as Lys1, Lys45, Arg10, Ser38 and the carboxy terminus are exposed to the interface between the two monomers in this dimer. As a result, several solvent and solute molecules such as acetate, phosphate ion, glycerol and several water molecule are bound here which further strengthen the interactions in this dimer (see below).

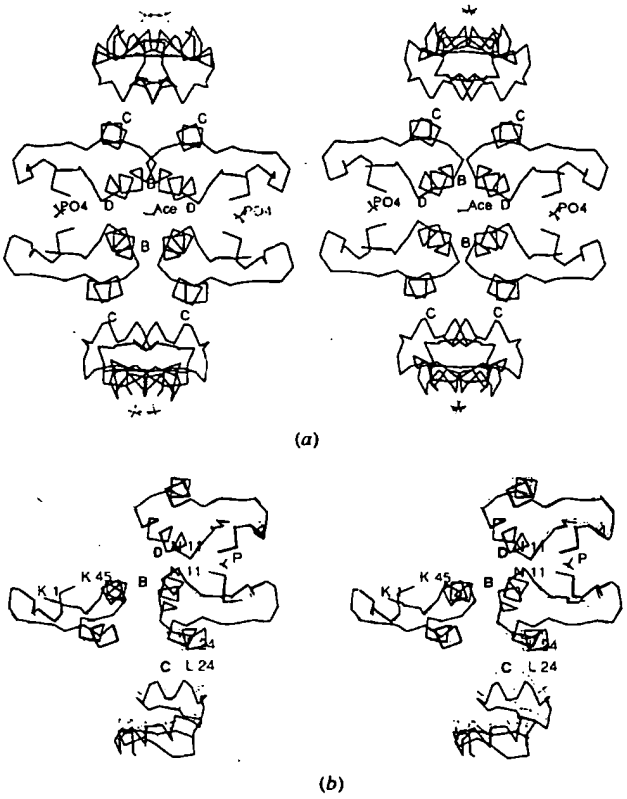

Fig. 9. (*a*) Stereo diagram of intermolecular interactions for α_1-PT viewed down the *b* axis (at $x = \frac{1}{2}$, $y = 0$). A twofold symmetry mate (along the *b* axis) for this 'dimer' shows a hydrophobic interaction of the second helices (*C*) resulting in a 'weak tetramer'. A diagonal twofold at ($x = \frac{1}{2}$, $y = 0$), operating on the *B* dimer, produces the polar-dimer interaction *D*. Association of dimers *B* and *D* is termed as a 'tight tetramer'. The solutes (phosphates and acetates) that bridge the polar dimer tightly are also shown. (*b*) Stereoview of $C\alpha$ atomic model of α_1-PT (light lines) and that of β-PT (dark lines) showing symmetry-related molecules involved in interactions *C* and *D*. One of the monomers of each purothionin is superimposed. Phosphate ions bound at the *D* interface are also shown. Note the small conformational changes of the backbone and expansion of the β-PT lattice at the *C* interface.

Table 5. *Interactions that stabilize the polar dimer in both α_1- and β-PT*

Atom from a monomer	Atom of a symmetry-related molecule	Distance in β-PT (Å)	Distance in α_1-PT (Å)
Asn11 OD1	Asn14' ND2	2.80	2.84
Thr7 O	Asn14' ND2	3.37	3.65
Asn11 ND2	Asn11' O	3.45	3.90
Asn11 ND2	Acetate	2.73	2.66
Lys1 NZ	Phosphate	Not observed	2.91
Lys45 OT	Water60 (101)*	2.71	3.47
Water60 (101)*	Asn14 ND2	3.10	3.33
Pro44 O	Phosphate	Not observed	3.27
Phosphate	Pro44' O	Not observed	3.27
Lys1 NZ	Water61'	2.80	Not observed
Water61'	Phosphate	3.00	Not observed
Ser2 OG	Water61'	2.88	Not observed
Ser38 OG	Water100' (79)*	2.61	3.0
GLC54(180) O1†	Ser2 OG	2.96	3.09
GLC54(180) O2†	Lys45' NZ	2.94	Not observed
GLC54(180) O2†	Glyn23 OE1	Not observed	2.88
GLC54(180) O3†	Tyr13 OH	3.15	3.36
GLC54(180) O3†	Arg17 NE	3.53	3.41
GLC55 O2	Asn14' O	2.95	Not observed
ACT52 O1	Asn11 ND2	2.73	3.01
ACT53 O2	Arg30' NH2	2.84	Not observed
ACT88 O2	Ser35 N	Not observed	2.87
GLC181 O2†	Lys1 N	Not observed	2.84
Sec-But OH	Thr7 OG1	Not observed	3.45
Arg10 CG	Arg10' CG	3.38	3.62

* Water60 and 100 in β-PT corresponds to water101 and 79 in α_1-PT, respectively.

† Glycerol (GLC) 54 in β-PT corresponds to GLC180 in α_1-PT. Glycerols 54 and 55 are in β-PT while 180 and 181 in α_1-PT.

3.3.2. *Tetramer association.* Various pairs of interactions may lead to higher order associations that may be relevant in solution. The tightest of these are the hydrophobic dimers (*B*) on two different levels, which make tetramer contacts through the polar dimer interaction *D* resulting in a compact tetramer (Fig. 9). This tetramer can also be thought of as a four-helix bundle (see Fig. 9), although the interaction between the helices is not of the conventional type (Cohen & Parry, 1990). The helices here are held by the already mentioned leucine ladders in one direction and with hydrogen bonds between aspargines in the perpendicular direction. This tetramer may be the building block or the seed for crystal growth of α_1- and β-PT.

3.3.3. *Crystal packing modification in β-PT.* Because of high sequence identity, the structures of α_1 and β-purothionins are similar. But the small sequence differences between β-PT and α_1-PT have significant effects on the crystal lattice. Such differences along with their consequences are discussed below.

Just as α_1-PT, β-PT crystallizes in the space group *I*422 and its crystal has a similar layer-like structure having the interactions A, *B*, *C*, *D* and *E* (Rao *et al.*, 1995). There is, however, a 3 Å difference in the *c*-dimension in β-PT crystals compared to α_1-PT. This difference has an important bearing on the packing relationship between molecules. Understanding this relationship in turn may aid in finding the causes of differences between the physical properties for the two purothionin crystals.

The difference in the *c* unit length can be explained in terms of variations in interlayer distances between the two crystals (see Fig. 9*b*). One of the monomers of each α_1- and β-purothionins are superimposed in this figure and corresponding symmetry-related molecules (related by interaction *C* and *D*) are generated. It can be seen from this figure that the monomers related by interaction *C* (β-PT molecules depicted in thick lines and α_1-PT molecules in thin lines) move apart in *c* direction and this movement is about 1.6 Å. The polar dimer *D* is almost identical in β-PT as compared to that in α_1-PT. Since there are two such instances for one unit cell, the net increase or expansion of the cell is about 3 Å in the *c* direction.

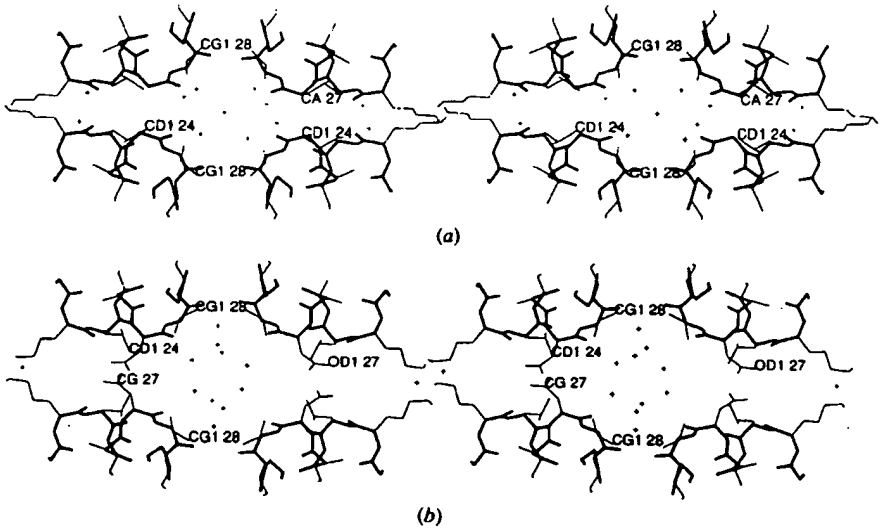

Fig. 10. Stereoviews of atomic model of the weak-tetramer (two *C* dimers related by twofold symmetry along the *c* axis in both α_1-PT and β-PT). Only *H*2 helices are shown. Backbone atoms are shown in thick lines while the side chains are shown in thin lines. Relevant atoms are labeled. (*a*) View for α_1-PT. Note the closeness of Cα of Gly27 and Cδ of Leu24. A single water molecule is packed in between four symmetry-related Val28 residues. (*b*) Similar view for β-PT. Note the expansion in *c* direction, thus making possible the packing of Asn27 side chain at the interface. Moreover, several water molecules are packed between Val28 residues.

Table 6. *Comparison of side-chain conformations between α_1- and β-PT*

	Conformation*									
	β-PT					α_1-PT				
Residue	χ_1	χ_2	χ_3	χ_4	χ_5	χ_1	χ_2	χ_3	χ_4	χ_5
Lys1	t	t	t	+		t	t	t	−	
Arg19	−	−	t	+	t	−	−	−	−	t
Lys23	−	t	t	−		−	−	t	t	
Arg30	−	t	+	+	t	−	t	t	t	t
Lys32	−	t	t	+		t	t	t	t	
Lys41	+	t	t	+		t	t	t	t	

* Abbreviations: t ≃ 180°; − ≃ −60°; + ≃ +60°.

The expansion of the *C* interface region as a result of Gly27→Asn27 change weakens interaction *C* further. This weakened *C* interaction explains several of the observed crystal properties. The β-PT crystals are more sensitive to mechanical strain than those of α_1-PT. They develop cracks along planes perpendicular to the *c* axis. Moreover, the crystals have a tendency to cleave along

The reasons for this cell expansion have their roots in a single residue change at position 27 (Gly to Asn). Fig. 10 illustrates this point. Fig. 10(*a*) shows a view of the weak tetramer in α_1-PT depicting the close contact between four symmetry-related Val28 residues with a single water packed between them. Moreover, there is a van der Waals contact between the side chain of Leu24 and the Cα atom of Gly27 of another molecule which makes interaction *C* with the former. Fig. 10(*b*) shows the corresponding view in β-PT. Here the side chain of Leu24 is in van der Waals contact with the side chain of Asn27 and not with the backbone Cα of this residue. Two monomers making this interaction are pushed apart to accommodate the side chain of Asn27 in between and more waters are added between protein molecules.

these planes and thin sections can easily be peeled off these crystals.

The polar dimer interactions in both the purothionins are listed in Table 5 for comparison. One important change in this dimer for β-PT is related to the formation of four side chain–backbone intermolecular stabilizing hydrogen bonds in addition to the two intermolecular hydrogen bonds between the side chains of Asn11 and Asn14 that exist in α_1-PT. The side chain–backbone hydrogen bonds exist between Asn14 ND2 and Thr7′ O and between Asn11 ND2 and Asn11′ O (see Table 5).

Despite the conformational changes of the side chains (Table 6), the backbone atoms of β-PT generally match well with those of α_1-PT (Fig. 6). The r.m.s. deviation between the positions of the backbone atoms of α_1- and β-purothionins is 0.36 Å whereas a difference of 0.94 Å is observed when all the homologous side chains are also included in the calculation.

There are, however, a few minor differences in the backbone of residues 33–37 of the floppy loop and in residues Arg19 and Gly20 of the turn between the helices. There is about a +20° difference in φ angles of these residues compared to those in α_1-PT. Moreover, the backbone atoms of these residues have an r.m.s. difference in their atomic positions of 0.51 Å compared to 0.36 Å for all the backbone atoms. This change in the backbone contributes to the altered conformation* for Arg19 (−, −, t, +) in β-PT rather than the (−, −, −, −) as seen in α_1-PT. As a result in β-PT, the guanidinium head of this residue is oriented towards the solvent cavity.

3.4. *New solute molecules essential for the lattice formation*

The new features of the structures that were not seen earlier are provided by the solute molecules found in both structures. All of these solute molecules are tightly bound through several contacts, the majority of them being electrostatic as well as polar. The fact that these solutes and solvents, especially phosphate and glycerol, which are located in the lattice even though they were absent in the crystallization medium, may have biological significance. Their presence may reflect the existence of a phospholipid-binding site which is important for the toxic effect exerted on the membrane.

3.4.1. *Phosphate-binding site.* In both α_1- and β-purothionins, there is a specific phosphate-binding site at the *D* interface discussed above. The polar dimer anchored by Asn11 and Asn14 results in a site with relatively high positive electrostatic potential. The site is formed at the center of the polar dimer due to the closeness of two symmetry-related Arg10 and two Lys1 residues and the N terminus (Figs. 2 and 9). In α_1-PT, the phosphate ion binds to symmetry-related pairs of NZ atoms of Lys1. Due to the small conformational changes caused by the different packing in β-PT, the phosphate ion causes a change in Lys1 conformation which makes the direct coordination impossible (see Fig. 6 and Table 5). Phosphate in β-PT is coordinated to two Lys1 through the water molecules.

3.4.2. *Glycerol-binding site.* Both α_1- and β-purothionins have a specific glycerol-binding site at the same location which is close to the phosphate-binding site. Glycerol is bound to the residues that outline the groove between the helical stem and the β-sheet arm (Figs. 2 and 6). In both structures the O3 of the glycerol molecule is bound to the hydroxyl O atom of Tyr13 and the NE of Arg17 whereas O1 is bound to Ser2 OG. There is, however, a difference between them as well. In β-PT, the O2 of glycerol molecule is hydrogen bonded to the symmetry-related Lys45, but in α_1-PT, the glycerol O2 atom interacts with Gln23. Thus, we see that the rearrangement of the unit cell and compaction of the *D* contact causes the glycerol molecule to change a partner in the interaction. The NZ atom of Lys45

* The notations used in this paper for the description of side-chain conformations are t = 180°, + = +60° and − = −60°. These definitions are the same as those used by Ponder & Richards (1987). The corresponding IUPAC convention is t = 180°, g^- = +60° and g^+ = −60°.

is more positively charged and a better donor for a hydrogen bond.

3.4.3. *Acetate-binding site.* Disordered acetate found in both α_1- and β-PTs is located in the four-helical bundle formed by four symmetry-related molecules. The presence of the acetate is reminiscent of the four-helix bundle of cytochrome *B* in which the heme group is found in the middle. Each disordered acetate forms hydrogen bonds to two symmetry-related Asn11 residues protruding toward the center (N Asn11 to O Ace, 2.7 Å). In such a location, it helps to neutralize this extremely positively charged molecule (+9) and contributes to the stability of the lattice.

We may conclude that without those negatively charged solute molecules (phosphate and acetate) placed on the symmetry elements it would be impossible to form the α_1-PT crystals. The intermolecular side-chain interactions which are responsible for the packing with their updated distances are listed in Table 5.

4. Summary

The structure determination of β-PT at higher resolution (1.7 Å) than α_1-PT (2.5 Å) has opened a new vista to understanding more about thionins in general. The initial failures in obtaining high-quality crystals of β-PT and then the difficulties in handling the crystals once they were obtained led us to not only look for crystal-stabilizing factors but also to look into the root causes of these crystal properties. Rerefinement of α_1-PT followed by the parallel refinement of β-PT in the final stages of α_1-PT refinement proved complementary and facilitated locating many critical solute particles in the lattice. The importance of the phosphate and acetate ions in stabilizing both crystals has thus been investigated.

Although the structure of β-PT has overall similarity to that of α_1-PT, there is a key difference between the two in crystal packing. The *c* dimension of the former is larger than that of the latter by 3 Å, and this expansion, originating from a change of residue 27 (Gly→Asn), has been attributed to the altered space between stacking layers of tetrameric molecules. Consequently, some of the side chains have different conformations and the crystals have different properties. The non-homologous amino acids are responsible for the toxins specificity toward different phospholipids (Garcia-Olmedo, Rodriquez-Palenzuela, Hernandez-Lucas, Ponz & Marana, 1989). Thus, the conformational changes might be pertinent to the modulation of biological activity. Modeling may clarify this difference further.

The finding that phosphate ions and glycerol molecules bind to purothionins has led us to believe that it may be relevant to biological activity of the thionins. It is reasonable to assume that those moieties are indicators of the phospholipid-binding site. Experimental support for this hyphothesis comes from Wada, Ozaki, Matsubara & Yoshizumi (1982) and Evans, Wang, Shaw & Vernon (1988). They showed that the toxins lose their toxicity when Tyr13 is iodinated. In our structure the glycerol molecule is bound to this crucial residue. The iodination would disrupt the glycerol and the phospholipid binding thus making the protein impotent towards membranes.

To elucidate the details of these important interactions we have carried out NMR experiments on binding inorganic phosphates and glycerol-3-phosphate and small phospholipid analogs (Markman *et al.*, 1992). Further the NMR three-dimensional structure determination of the α_1-PT–glycerol-3-phosphate complex is in process. The results, which will be presented elsewhere, indicate that this site is indeed the phospholipid binding site. Thus, although preliminary, these results seem to support the crystallographic finding suggesting that the phosphate-binding site is not an artifact of the symmetry of the crystal lattice, but indeed related to the toxicity of these proteins.*

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* Atomic coordinates have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1BHP). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: GR0393). At the request of the authors, the atomic coordinates will remain privileged until 15 March 1996.

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NiceProt View of TrEMBL: Q43205

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**Q43205_WHEAT**
Primary accession number	**Q43205**
Secondary accession numbers	None
Entered in TrEMBL in	Release 01, November 1996
Sequence was last modified in	Release 01, November 1996
Annotations were last modified in	Release 26, March 2004

Name and origin of the protein

Protein name	**Alpha-1 purothionin**
Synonyms	None
Gene name	None
From	Triticum aestivum (Wheat) [TaxID: 4565]
Taxonomy	Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae; Triticeae; Triticum.

References

[1] NUCLEOTIDE SEQUENCE.
Inagaki A., Matsuoka Y., Tsunewaki K.;
Submitted (APR-1996) to the EMBL/GenBank/DDBJ databases.

Comments

- ***SIMILARITY***: Belongs to the plant thionin (TC 1.C.44) family [view classification].

Cross-references

EMBL	D84390; BAA12336.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P01543; 1BHP. [HSSP ENTRY / PDB]
GO	GO:0006952; Biological process: defense response *(inferred from electronic annotation).* QuickGo view.
InterPro	IPR001010; Thionin. Graphical view of domain structure. PF00321; Thionin; 1.

Pfam	Pfam graphical view of domain structure.
PRINTS	PR00287; THIONIN.
PROSITE	PS00271; THIONIN; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
ProtoMap	Q43205.
PRESAGE	Q43205.
ModBase	Q43205.
SMR	Q43205; B4018F414E226B9F.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Plant defense; **Thionin**.

Features

None

Sequence information

Length: **136 AA** Molecular weight: **14542 Da** CRC64: **B4018F414E226B9F** [This is a checksum on the sequence]

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        10         20         30         40         50         60
MGSKGLKGVM VCLLILGLVL EQVQVEGKSC CRTTLGRNCY NLCRSRGAQK LCSTVCRCKL

        70         80         90        100        110        120
TSGLSCPKGF PKLALESNSD EPDTIEYCNL GCRSSVCDYM VNAAADDEEM KLYVENCGDA

       130
CVNFCNGDAG LTSPDA
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Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

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US006329011B1

(12) **United States Patent**
Oita

(10) **Patent No.: US 6,329,011 B1**
(45) **Date of Patent: Dec. 11, 2001**

(54) **ANTIMICROBIAL AGENT AGAINST ACID-RESISTANT AND HEAT-RESISTANT BACTERIA**

(75) Inventor: **Shigeru Oita**, Zentsuji (JP)

(73) Assignees: **Director General of Shikoku National Agricultural Experiment Station; Ministry of Agriculture, Forestry and Fisheries**, both of Zentsuji (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/680,403**

(22) Filed: **Oct. 5, 2000**

(30) **Foreign Application Priority Data**

Jul. 26, 2000 (JP) 12-224738

(51) **Int. Cl.**[7] **A61K 38/00**; C07K 14/00; A23L 2/02

(52) **U.S. Cl.** **426/599**; 514/12; 530/324; 426/599; 426/656; 426/335; 426/268; 435/252; 435/252.1; 435/252.31; 435/252.5; 435/832; 435/833; 435/834; 435/835; 435/836; 435/837; 435/838; 435/839

(58) **Field of Search** 514/2, 12; 530/324; 426/268, 321, 656, 335, 599; 435/252.5, 252, 252.1, 252.31, 832, 833, 834, 835, 836, 837, 838, 839

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* cited by examiner

Primary Examiner—Christopher S. F. Low
Assistant Examiner—Chih-Min Kam
(74) *Attorney, Agent, or Firm*—Oblon, Spivak, McClelland, Maier & Neustadt, P.C.

(57) **ABSTRACT**

An antimicrobial agent with a high degree of safety is provided, which is derived from a natural product and can exhibit growth-inhibitory activity against acid-resistant and heat-resistant bacteria such as *Alicyclobacillus acidoterrestris*, which is resistant against pasteurization and causes spoilage of fruit juice. The antimicrobial agent against acid-resistant and heat-resistant bacteria contains as an effective ingredient alpha-type thionin and/or beta-type thionin. A preservative for fruit juice is also provided, which contains as an effective ingredient the alpha-type thionin and/or beta-type thionin.

15 Claims, 1 Drawing Sheet

FIG. 1A

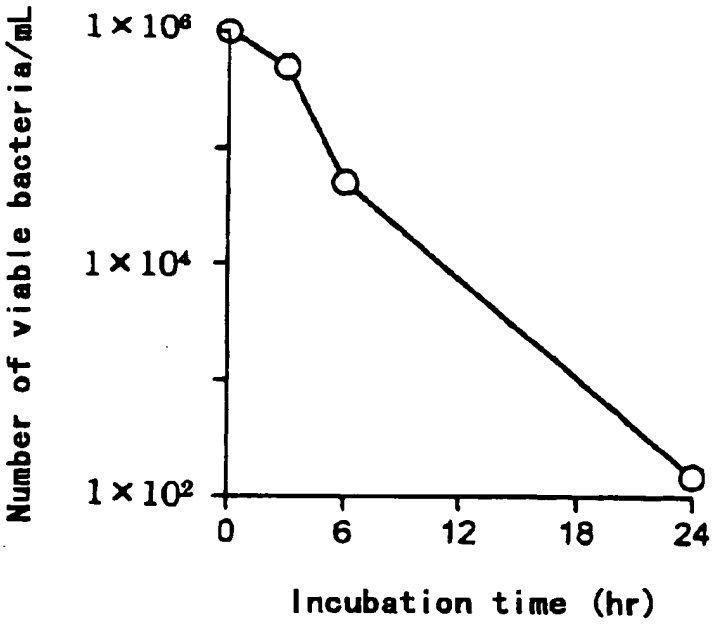
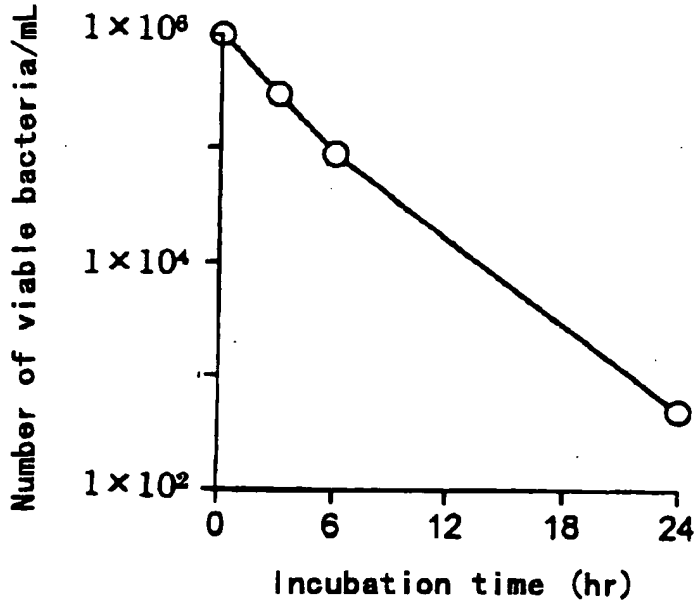

Number of viable bacteria/mL
1×10⁶
1×10⁴
1×10²
0
6
12
18
24
Incubation time (hr)

FIG. 1B


Number of viable bacteria/mL
1×10⁶
1×10⁴
1×10²
0
6
12
18
24
Incubation time (hr)

ANTIMICROBIAL AGENT AGAINST ACID-RESISTANT AND HEAT-RESISTANT BACTERIA

FIELD OF THE INVENTION

The present invention relates to an antimicrobial agent against acid-resistant and heat-resistant bacteria, and more specifically, to an antimicrobial agent, which contains as an effective ingredient a safe peptide derived from a natural product, against acid-resistant and heat-resistant bacteria such as *Alicyclobacillus acidoterrestris*.

BACKGROUND OF THE INVENTION

As typical bacteria, which exhibit acid resistance in combination with heat resistance, there are known *Alicyclobacillus acidoterrestris*, *Alicyclobacillus acidocaldarius*, etc. The spores of these bacteria possess resistance against the normally employed pasteurization method for fruit juice. Therefore, in recent years, the spoilage of fruit juice caused by *Alicyclobacillus acidoterrestris* has been a serious problem throughout the world.

In order to suppress growth of the bacterium in fruit juice, it is effective to add a synthetic preservative such as benzoic acid. However, there is now strongly demanded a material with an enhanced degree of safety, which is derived from a natural product but not from a synthetic product.

As an antimicrobial agent, which is derived from a natural product and is effective against the bacterium, there has been reported only nisin, which is a peptide derived from lactic acid bacterium (International Journal of Food Science and Technology, vol. 34, pp. 81 to 85, 1999). However, nisin contains a special type of amino acids such as dehydroalanine.

Consequently, for the preservation and storage of fruit juice, there is demanded an antimicrobial agent, which is derived from a natural product and is effective at lower concentration, against acid-resistant and heat-resistant bacteria such as *Alicyclobacillus acidoterrestris*.

The inventor of the present invention has studied characteristics of alpha-type thionin and beta-type thionin, which are peptides derived from wheat and barley. To the best of the inventor's knowledge, no one has reported any antimicrobial activity of thionins against *Alicyclobacillus acidoterrestris*, which is an acid-resistant and heat-resistant bacteria, while its antimicrobial activity against phytopathogenic fungi has previously been known (Plant Science, vol. 92, pp. 169 to 177, 1993). In addition, such thionins do not contain any special amino acid at all, unlike the above-mentioned nisin derived from lactic acid bacteria.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an antimicrobial agent with an enhanced degree of safety, which is derived from a natural product and exhibits growth-inhibitory activity against *Alicyclobacillus acidoterrestris*, which has acid-resistance and heat-resistance and causes spoilage of fruit juice. Another object of the present invention is to provide a preservative for fruit juice, which comprises the antimicrobial agent described above.

In order to attain the above-mentioned objects, the inventor of the present invention has conducted intensive screening of antimicrobial substances against *Alicyclobacillus acidoterrestris* from various agricultural crops, with the result that they found that alpha-type thionin and beta-type thionin, which are peptides of wheat and barley, exhibit growth-inhibitory activity against this bacteria. Thus, the inventor of the present invention has completed the present invention based on this finding.

Briefly, the present invention relates to an antimicrobial agent against acid-resistant and heat-resistant bacteria, characterized in that the antimicrobial agent contains as an effective ingredient alpha-type thionin and/or beta-type thionin.

Further, the present invention relates to a preservative for fruit juice, characterized in that the preservative contains as an effective ingredient alpha-type thionin and/or beta-type thionin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B show changes with a lapse of time in number of viable bacteria of *Alicyclobacillus acidoterrestris* in each of fruit juice with thionin added; in which FIGS. 1A and 1B indicate results obtained from orange juice and apple juice, respectively.

DETAILED DESCRIPTION OF THE INVENTION

Thionins, which are usable in the present invention, can be obtained from flour of grains such as barley, wheat, oats and rye, through extraction with saline solution or acids such as hydrochloric acid, sulfuric acid and acetic acid, and in addition, can also be produced with use of recombinant microorganisms or plants containing thionin genes.

Both of alpha-type thionin and beta-type thionin are composed of about 45 amino acids and about 8 cysteine contained among them, and have the molecular structure characterized in that partial or every cysteine residues form cross-linking by the disulfide bonds. The values of pH in fruit juices are usually in the range of 3 to 4 and these thionins are not denatured even in such acidic condition.

Such thionins can be purified by concentrating an extract of barley or wheat through salting out with ammonium sulfate, etc., followed by high-performance liquid chromatography, but any mixture (crude purification products) produced in the course of such purification process, can similarly be used in the present invention, only in the case where they can exhibit the desired antimicrobial activity.

The process for extraction and purification of thionins from barley was for example described in Planta, vol. 176, pp. 221 to 229 (1988), and it is also possible to carry out extraction and purification from other varieties of wheat and barley in accordance with the process.

There have already been known the entire amino acid sequences of thionins produced from barley, wheat, and oats (Plant Molecular Biology, vol. 26, pp. 25 to 37, 1994) as well as the amino acid composition of thionin of rye (Journal of Agricultural and Food Chemistry, vol. 26, pp. 794 to 796, 1978). It is to be noted that depending upon the race of wheat and barley, there may exist variant peptides having one or several amino acid residues undergone replacement, addition or deletion as compared with the known amino acid composition and sequences, and such variant peptides are included in the thionins which are usable in the present invention, as long as they exhibit the objective antimicrobial activity.

In the case where thionin is used as an antimicrobial agent against acid-resistant and heat-resistant bacteria or as a preservative for fruit juice, thionin or crude thionin is desirably added to fruit juice to a final concentration ranging

from 5 to 100 μg/mL in case of the purified alpha-type one or ranging from 10 to 100 μg/mL in case of the purified beta-type one, in order to suppress growth of acid-resistant and heat-resistant bacteria such as *Alicyclobacillus acidoterrestris*, in fruit juice.

Since thionin does not lose antimicrobial activity after being heated under acidic conditions at 100° C. for 10 min. (Agricultural and Biological Chemistry, vol. 34, pp. 1089 to 1094, 1970), the antimicrobial activity of thionin can be maintained even when thionin is added to fruit juice, followed by pasteurization.

From the fact that no report has yet been published on any thionin-resistant mutant microorganism, and that thionin is degraded rapidly by digestive enzymes such as trypsin (Journal of the Japanese Society for Food Science and Technology, vol. 47, pp. 423 to 429, 2000), its effect on the enterobacteria can be considered to be extremely minor. In addition, it has been reported that one oral administration of thionin to guinea pigs at a dose of 103 to 229 mg/kg body weight, followed by observation for 7 days, did not result in detection of any abnormalities (Cereal Chemistry, vol. 19, pp. 301 to 307, 1942).

According to the present invention, there is provided the antimicrobial agent with a high degree of safety, which contains thionin as an effective ingredient and is derived from a natural product. Furthermore, the present invention also provides the preservative for fruit juice, which contains thionin as an effective ingredient. Such the antimicrobial agent and preservative exhibit growth inhibition against acid-resistant and heat-resistant bacteria such as *Alicyclobacillus acidoterrestris*, which cause spoilage of fruit juice. In addition, thionin is not denatured even after being heated under acidic conditions, and therefore effective for prevention of spoilage of fruit juice. Aqueous solutions of thionins are clear and odorless, and consequently do not affect the flavor of fruit juice.

EXAMPLES

The present invention will be described in detail with reference to examples, but the present invention is not intended to limit thereto.

Production Example 1

Grains of hull-less barley (variety: "ICHIBAN-BOSHI") were milled and powdered by a cyclon mill, and 100 g of the powders were admixed with 300 mL of distilled water, followed by stirring for 1 hour at 4° C. and subjecting to centrifugation to remove the resultant supernatant.

Then, the precipitate was admixed with 200 mL of 1M aqueous sodium chloride solution, and the mixture was stirred for 2 hours at 4° C. and subjected to centrifugation. The resultant supernatant was subjected with ammonium sulfate (50 to 90% saturated), and the recovered precipitate was suspended in a phosphate buffer, followed by centrifugation. Thus obtained supernatant was subjected to high-performance liquid chromatography to obtain purified alpha-type thionin and beta-type thionin of barley. In high-performance liquid chromatography, Wakosil 5C4-200, 4.6 mmϕ×250 mm (supplied by Wako Pure Chemicals Ind. of Japan) was employed as a column, and concentration-gradient elution was carried out with water (pH 2.1) containing 0.1% trifluoroacetic acid and 0→40% (0→40 min) acetonitrile at a flow rate of 0.5 mL/min. Then, the collected fractions were evaporated to dryness with a centrifugal evaporator, followed by an amino acid analysis and a mass spectrum analysis, thereby being identified as alpha-type thionin and beta-type thionin. There were yielded 37 mg of alpha-type thionin and 12 mg of beta-type thionin, respectively, in terms of 1 kg of powdered grains of "ICHIBAN-BOSHI".

Production Example 2

100 g of commercially available, soft wheat flour was admixed with 300 mL of 0.15 N hydrochloric acid, and the resultant mixture was stirred. The mixture was then settled for 30 minutes at 37° C. and stirred again, followed by centrifugation. The resultant supernatant was neutralized by dropwise adding 10 N of aqueous sodium hydroxide solution, and then centrifugation was conducted again.

The supernatant thus obtained was subjected with ammonium sulfate (50 to 90% saturated), and the recovered precipitate was suspended in a phosphate buffer. The supernatant separated out by centrifugation was subjected to high-performance liquid chromatography under the same conditions as in Production Example 1, to give purified alpha-type thionin of wheat.

The collected fractions were concentrated to dryness by a centrifugal evaporator, followed by amino acid analysis and mass spectrum analysis, thereby being identified as alpha-type thionin. There was yielded 40 mg of alpha-type thionin in terms of 1 kg of soft wheat flour.

Example 1

Alicyclobacillus acidoterrestris strain ATCC 49025 was inoculated into YPGB culture medium (0.25% of yeast extract, 0.5% of polypeptone, 0.1% of D-glucose, 0.05% of magnesium sulfate 7 hydrate ($MgSO_4.7H_2O$), 0.2% of potassium chloride, pH 4), followed by cultivation for 2 days at 37° C., and the cultured broth was treated for 1 hour at 60° C. to prepare a spore suspension.

The YPGB culture media, which contained the specified amount of each different thionin as obtained in Production Examples 1 and 2, were inoculated individually with the spore suspension at a rate of 1×10^4 spores/mL, and cultivation was conducted for 2 days at 37° C., followed by investigation for bacterial growth.

The results are shown in Table 1. In the investigation, the bacterial growth was judged on the basis of visual observation of the degree of turbidity of each culture medium, but in the cases of 50 μg/mL and 100 μg/mL in thionin addition amount, the bacterial growth was judged through the plate culture of diluted broth, because the culture media became turbid by thionin. Further, in the case where thionins were not added as controls, bacterial growth was observed in every test group.

TABLE 1

	Bacterial growth Thionin concentration (μg/mL)						
Type of thionins	100	50	20	10	5	2	1
Alpha type of barley	-	-	-	-	-	+	+
Alpha type of wheat	-	-	-	-	-	+	+
Beta type of barley	-	-	-	-	+	+	+

+: bacterial growth was observed
-: bacterial growth was not observed

Example 2

Commercially available, 100% orange juice and apple juice (pH value of 3.6 for both juice) were added with

alpha-type thionin of barley at a final concentration of 20 μg/mL and then inoculated, at a rate of 1×10^6 spores/mL, with the spore suspension of *Alicyclobacillus acidoterrestris* strain ATCC 49025 prepared in Example 1, respectively, and were incubated at 37° C. The number of viable bacteria in each juice was counted with a lapse of time though the dilution plate culture (for 2 days at 37° C.) in a potato dextrose agar medium (supplied by Nissui Seiyaku Co. of Japan). The results shown in FIGS. 1A and 1B are obtained. In the figures, FIG. 1A indicates the result obtained from orange juice and FIG. 1B, from apple juice, respectively.

As apparent from the figures, the number of viable bacteria of *Alicyclobacillus acidoterrestris* decreased drastically with a lapse of time, leading to the conclusion that the effect of addition of alpha-type thionin of barley was confirmed.

What is claimed is:

1. A fruit-juice comprising a thionin.

2. The fruit juice of claim 1, wherein said thionin is selected from the group consisting of an alpha-type thionin and a beta-type thionin.

3. The fruit juice of claim 1, wherein said thionin is an alpha-type thionin in an amount ranging from 5 to 100 μg/mL.

4. The fruit juice of claim 1, wherein said thionin is a beta-type thionin in an amount ranging from 10 to 100 μg/mL.

5. The fruit juice of claim 1, wherein said fruit juice has been pasteurized.

6. The fruit juice of claim 1, wherein said fruit juice is apple juice.

7. The fruit juice of claim 1, wherein said fruit juice is orange juice.

8. The fruit juice of claim 1, wherein said thionin is a barley, oats, rye or wheat thionin.

9. A method for preserving a fruit juice or retarding the spoilage of a fruit juice comprising adding an amount of a thionin to said fruit juice effective to preserve said fruit juice or retard the spoilage of said fruit juice.

10. The method of claim 9, wherein said thionin is selected from the group consisting of an alpha-type thionin and a beta-type thionin.

11. The method of claim 9, wherein said fruit juice contains an acid-resistant or heat-resistant bacterium.

12. The method of claim 9, wherein said fruit juice contains *Alicyclobacillus acidoterrestris*.

13. A method of inhibiting the growth of an acid-resistant or heat-resistant bacteria belonging to the genus Alicyclobacillus, comprising contacting said bacterium with an effective amount of a thionin.

14. The method of claim 13, wherein said thionin is selected from the group consisting of alpha-type thionin and beta-type thionin.

15. The method of claim 13, wherein said thionin is a barley, oats, lye or wheat thionin.

* * * * *

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Search Results - Record(s) 1 through 4 of 4 returned.

☐ 1. 20030091555. 04 Feb 02. 15 May 03. Bactericidal composition containing peptide and chelating agent. Oita, Shigeru. 424/94.63; 514/564 A61K038/48 A61K031/195.

☐ 2. 6329011. 05 Oct 00; 11 Dec 01. Antimicrobial agent against acid-resistant and heat-resistant bacteria. Oita; Shigeru. 426/599; 426/268 426/335 426/656 435/252 435/252.1 435/252.31 435/252.5 435/832 435/833 435/834 435/835 435/836 435/837 435/838 435/839 514/12 530/324. A61K038/00 C07K014/00 A23L002/02.

☐ 3. US20030091555A. Bacterial composition used for sterilizing food poisoning bacteria, includes ethylenediaminetetraacetic acid or its metal salts and alpha- or beta-type thionin. OITA, S. A61K031/195 A61K031/198 A61K038/00 A61K038/48 A61P001/02 A61P031/04.

☐ 4. US 6329011B. Fruit juice contains thionine derived from natural product as preservative. OITA, S. A01N037/46 A01N043/72 A01N043/84 A01N065/00 A23L002/02 A23L002/42 A23L002/44 A23L003/3472 A23L003/3526 A61K038/00 C07K014/00.

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Terms	Documents
L2 and ($thionin or thionin$)	4

Prev Page Next Page Go to Doc#

First Hit

L3: Entry 1 of 4 File: PGPB May 15, 2003

PGPUB-DOCUMENT-NUMBER: 20030091555
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030091555 A1

TITLE: Bactericidal composition containing peptide and chelating agent

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Oita, Shigeru	Zentsuji-shi		JP	

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	COUNTRY	TYPE CODE
NATIONAL AGRICULTURAL RESEARCH ORGANIZATION	Tsukuba-shi		JP	03

APPL-NO: 10/ 067124 [PALM]
DATE FILED: February 4, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2001-251048	2001JP-2001-251048	August 22, 2001

INT-CL: [07] A61 K 38/48, A61 K 31/195

US-CL-PUBLISHED: 424/94.63; 514/564
US-CL-CURRENT: 424/94.63; 514/564

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

A bactericidal composition is provided, which comprises as effective ingredients (a) at least one substance selected from the group consisting of ethylenediaminetetraacetic acid and metal salts thereof and (b) at least one substance selected from the group consisting of alpha-type thionin and beta-type thionin. The bactericidal composition, for example, has the effect of sterilizing food poisoning bacteria at a low concentration and is highly safe.

WEST Search History

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DATE: Tuesday, March 01, 2005

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	DB=USPT; PLUR=YES; OP=AND		
☐	L1	6329011.pn.	1
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND		
☐	L2	oita.in.	372
☐	L3	L2 and ($thionin or thionin$)	4

END OF SEARCH HISTORY

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SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2005/Feb W3
         (c) format only 2005 The Dialog Corp.
*File 155: Medline has been reloaded; acession numbers have changed.
Please see HELP NEWS 154.
  File 654:US Pat.Full. 1976-2005/Feb 24
         (c) Format only 2005 The Dialog Corp.
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  File 349:PCT FULLTEXT 1979-2002/UB=20050217,UT=20050210
         (c) 2005 WIPO/Univentio
  File 340:CLAIMS(R)/US Patent 1950-05/Feb 24
         (c) 2005 IFI/CLAIMS(R)
*File 340: 2004 Reload is online as of October 6, 2004.  Pricing
changes effective October 1, 2004.  See HELP NEWS 340 for details.
  File   5:Biosis Previews(R) 1969-2005/Feb W3
         (c) 2005 BIOSIS
*File   5: Price change effective Jan 1, 2005. Enter HELP
RATES 5 for details.
  File 348:EUROPEAN PATENTS 1978-2005/Feb W03
         (c) 2005 European Patent Office
  File 347:JAPIO Nov 1976-2004/Oct(Updated 050208)
         (c) 2005 JPO & JAPIO
*File 347: JAPIO data problems with year 2000 records are now fixed.
Alerts have been run.  See HELP NEWS 347 for details.
  File  65:Inside Conferences 1993-2005/Feb W3
         (c) 2005 BLDSC all rts. reserv.
  File  35:Dissertation Abs Online 1861-2005/Feb
         (c) 2005 ProQuest Info&Learning
  File 342:Derwent Patents Citation Indx 1978-05/200510
         (c) 2005 Thomson Derwent
  File 203:AGRIS 1974-2004/Nov
         Dist by NAL, Intl Copr. All rights reserved
  File 156:ToxFile 1965-2005/Feb W3
         (c) format only 2005 The Dialog Corporation
*File 156: Updating of ToxFile has resumed, with
UD=20041205.
  File  94:JICST-EPlus 1985-2005/Jan W2
         (c)2005 Japan Science and Tech Corp(JST)
  File 398:Chemsearch 1957-2005/Jan
         (c) 2005 Amer.Chem.Soc.
*File 398: Use is subject to the terms of your user/customer agreement.
Problems with SORT.  RANK charge added. See HELP RATES 398.
  File 357:Derwent Biotech Res. _1982-2005/Feb W4
         (c) 2005 Thomson Derwent & ISI
  File  73:EMBASE 1974-2005/Feb W3
         (c) 2005 Elsevier Science B.V.
*File  73: Price change effective Jan 1, 2005. Enter HELP
RATES 73 for details.
  File  50:CAB Abstracts 1972-2005/Jan
         (c) 2005 CAB International

      Set  Items  Description
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Cost is in DialUnits
?ds

Set     Items   Description
S1        373   ALPHA (2N) THIONIN?
S2        313   RD (unique items)
S3     279736   'EDTA' OR 'EDTA (3-)' OR 'EDTA ACTION' OR 'EDTA ADDITION'
S4      53755   'EDTA'
S5      44607   R1-R4
S6         79   S2 AND (S3 OR S4 OR S5 OR EDTA? OR ETHYLENEDIAMINE?)
S7         51   S6/2002:2005
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S8 28 S6 NOT S7
S9 689 E3-E33
S10 1 'PUROTHIORINS'
S11 689 S9 OR S10
S12 128 S11 AND (EDTA? OR ETHYLENE? OR ETHYLENEDIAMINE? OR EDTA)
S13 50 S12/2002:2005
S14 78 S12 NOT S13
S15 70 S14 NOT S8
?t s15/9/1

15/9/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08856644 PMID: 2610351
Histochemical localization of cysteine-rich proteins by tissue printing on nitrocellulose.
Pont-Lezica R F; Varner J E
Department of Biology, Washington University, St. Louis, Missouri 63130.
Analytical biochemistry (UNITED STATES) Nov 1 1989, 182 (2) p334-7, ISSN 0003-2697 Journal Code: 0370535
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A rapid technique for the histochemical localization of cysteine-rich proteins in plant tissues was developed. It is based on the immediate transfer of proteins to nitrocellulose membranes when a fresh cut organ is pressed against the membrane surface. The print was labeled for cysteine-rich proteins by reduction and alkylation of cysteinyl residues with dansylated iodoacetamide [N-iodoacetyl-N'-(-5-sulfo-1-naphthyl) **ethylenediamine**]. The S-carboxymethylated proteins were visualized by their fluorescence when excited with 360 nm light.
Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Descriptors: *Collodion; *Cysteine--analysis--AN; *Histocytochemistry --methods--MT; *Proteins--analysis--AN; Hordeum--analysis--AN; Lectins --analysis--AN; Membranes, Artificial; Naphthalenesulfonates; Oxidation-Reduction; Plant Lectins; Plant Proteins--analysis--AN; Potatoes --analysis--AN
CAS Registry No.: 0 (Lectins); 0 (Naphthalenesulfonates); 0 (Plant Lectins); 0 (Plant Proteins); 0 (Proteins); 36930-63-9 (1,5-I-AEDANS); 52-90-4 (Cysteine); 9004-70-0 (Collodion); 9009-72-7 (purothionin)
Record Date Created: 19900222
Record Date Completed: 19900222
?t s15/3,kwic/2-70
>>>KWIC option is not available in file(s): 398, 399

15/3,KWIC/2 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004925643
Derwent Accession: 2002-010726
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin
Inventor: Scott Waldman, INV
Jason Park, INV
Stephanie Schulz, INV
Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ, MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia, PA, 19103, US

Publication Number	Kind	Date	Application Number	Filing Date

Main Patent	US 20010036635	A1	20011101	US 2001819247	20010327
Provisional				US 60-192229	20000327

Fulltext Word Count: 30220

Summary of the Invention:
...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein- specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/3 (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004918035
Derwent Accession: 2002-381264
Compositions and methods for identifying and targeting cancer cells of alimentary canal origin
Inventor: Scott Waldman, INV
Jason Park, INV
Stephanie Schulz, INV
Correspondence Address: Mark DeLuca, Esq. WOODCOCK WASHBURN KURTZ, MACKIEWICZ & NORRIS LLP One Liberty Place - 46th Floor, Philadelphia, PA, 19103, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20010029019	A1	20011011	US 2001819249	20010327
Provisional				US 60-192229	20000327

Fulltext Word Count: 30022

Summary of the Invention:
...any material capable of binding proteins. Well-known solid phase

fluorouracil, melphalan, chlorambucil, cyclophosphamide, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...

15/3,KWIC/9 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4384858
Derwent Accession: 1999-180474
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; IN VITRO METHOD OF DIAGNOSING METASTASIZED COLORECTAL CANCER BY DETECTING GENE EXPRESSION OF COLORECTAL CANCER-ASSOCIATED TRANSCRIPT-1, AN ALTERNATE FORM OF HEAT-STABLE TOXIN RECEPTOR, IN CELLS OF SAMPLE
Inventor: Waldman, Scott A., Ardmore, PA
Pearlman, Joshua M., Philadelphia, PA
Barber, Michael T., Paoli, PA
Schulz, Stephanie, West Chester, PA
Parkinson, Scott J., Philadelphia, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Eyler, Yvonne (Art Unit: 162)
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6120995	A	20000919	US 97908643	19970807

Fulltext Word Count: 34307

Description of the Invention:
...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens

15/3,KWIC/10 (Item 9 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4118486
Derwent Accession: 1995-178646
Utility
CERTIFICATE OF CORRECTION
C/ **Methods of treating metastatic colorectal cancer with ST receptor binding compounds**
; **RADIOACTIVE THERAPEUTIC AGENT, RECEPTOR BINDING MOIETY**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Green, Lora M. (Art Unit: 168)
Assistant Examiner: Ricigliano, Joseph W.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5879656	A	19990309	US 96583447	19960105
CIP	US 5518888	A	19960521	US 93141892	19931026

Fulltext Word Count: 33695

Description of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...hours at room temperature in 0.4M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and... activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

Non-exemplary or Dependent Claim(s):
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/16 (Item 15 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4085182
Derwent Accession: 1997-480228
Utility
C/ **Alteration of amino acid compositions in seeds**
Inventor: Jung, Rudolf, Des Moines, IA
Hastings, Craig, Perry, IA
Coughlan, Sean, Des Moines, IA
Hu, David, Johnston, IA
Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA
Pioneer Hi-Bred International Inc (Code: 17947)
Examiner: LeGuyader, John (Art Unit: 165)
Assistant Examiner: McGarry, Sean
Law Firm: Pioneer Hi-Bred International, Inc.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5850016	A	19981215	US 96618911	19960320

Fulltext Word Count: 12377
Description of the Invention:
...invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (1976); incorporated herein in its... pH 5.2 and concentrated in the dialysis bags to about 100 ml with dry **polyethyleneglycol** (PEG 8000). Precipitated contaminating globulin proteins are removed by centrifugation at 6000Xg for 15 min...

15/3,KWIC/17 (Item 16 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4057665
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; **ANTIFUNGAL, ISOLATED FROM PLANT SEEDS**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P.A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R.G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: McElwain, Elizabeth F. (Art Unit: 169)
Combined Principal Attorneys: Thomson, Marian T.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5824869	A	19981020	US 96777192	19961227
Division	US 5689043	A		US 95452078	19950526
Division	US 5538525	A		US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/20 (Item 19 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3913150
Derwent Accession: 1994-183512
Utility
C/ **DNA encoding biocidal proteins**
; **DNA SEQUENCES IN PROTEINS AND VECTORS IN CELLS**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Berkshire, GB England
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Hendricks, Keith D. (Art Unit: 184)
Law Firm: Cushman Darby & Cushman Intellectual Property Group of Pillsbury Madison & Sutro LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5691199	A	19971125	US 95451566	19950526
Division	US 5514779	A		US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 14086

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5, 5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/21 (Item 20 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3910716 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; **GENETIC ENGINEERING**
Inventor: De Bolle, Miguel, Louvain, BE

Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)
Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5689048	A	19971118	US 95471329	19950602
Division	US 5482928	A		US 93117080	19931220
Priority				GB 915052	19910311
				GB 915684	19910319

Fulltext Word Count: 6067

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...

...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (.circle-solid...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/22 (Item 21 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3910711
Derwent Accession: 1993-100978

Utility
C/ **Biocidal proteins**
; **GENETIC ENGINEERING**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P.A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R.G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)
Assistant Examiner: McElwain, Elizabeth F.
Law Firm: Cushman Darby & Cushman Intellectual Property Group of Pillsbury Madison & Sutro, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5689043	A	19971118	US 95452078	19950526
Division	US 5538525	A		US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 11738

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

Assistant Examiner: McElwain, Elizabeth F.
Law Firm: Cushman Darby & Cushman, L.L.P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5538525	A	19960723	US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829

Fulltext Word Count: 11737

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor(Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/25 (Item 24 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3725121
Derwent Accession: 1995-178646
Utility
C/ **ST receptor binding compounds and methods of using the same ; IMAGING METASTASIZED COLORECTAL CANCER, TOXTIN PEPTIDES LESS THAN 25 UNITS**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Scheiner, Toni R. (Art Unit: 182)
Assistant Examiner: Green, Lora M.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5518888	A	19960521	US 93141892	19931026

Fulltext Word Count: 28768

Summary of the Invention:

...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:

...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2... hours at room temperature in 0.4M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]113 In-DTPA is ...

15/3,KWIC/26 (Item 25 from file: 654)

DIALOG(R)File 654:US Pat.Full.

3720578

Derwent Accession: 1994-183512

Utility

C/ **Biocidal proteins from plants**

; **CHITINASES, FUNGICIDES**

Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Forest Park, GB England
Vanderleyden, Jozef, Heverlee, BE

Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)

Examiner: Wax, Robert A. (Art Unit: 184)

Assistant Examiner: Hendricks, Keith D.

Law Firm: Cushman Darby & Cushman

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5514779	A	19960507	US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 13401

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/27 (Item 26 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3685299 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
Inventor: De Bolle, Miguel, Leuven, BE
Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Imperial Chemical Industries PLC(03), London, GB, England
Imperial Chemical Industries Ltd GB (Code: 41248)
Examiner: Furman, Keith C. (Art Unit: 184)
Law Firm: Cushman, Darby & Cushman

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5482928	A	19960109	US 93117080	19931220
PCT	WO 9215691		19920917	WO 92GB423	19920310
		371:	19931220		
		102e:	19931220		
Priority				GB 915052	19910311
				GB 915684	19910319

Fulltext Word Count: 6028

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...agglutinin or UDA (Broekaert, W. F. et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C. et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...previously described (Peumans, W. J. et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, D. G. and Fisher, N...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...
...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and

from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (. . .), after 60...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/28 (Item 27 from file: 654)

3181895
Derwent Accession: 1989-220454
Utility
EXPIRED
C/ **Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations**
Inventor: Pigiet, Vincent P., Neshanic Station, NJ
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Page, Thurman K. (Art Unit: 152)
Assistant Examiner: Rucker, Susan S.
Law Firm: Saliwanchik & Saliwanchik

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5028419	A	19910702	US 89397802	19890823
Division	US 4894223	A		US 88140353	19880104
CIP	US 4919924	A		US 85770498	19850828
CIP	Abandoned			US 84674893	19841126

Disclaimer Date: 20070424

Fulltext Word Count: 3722

Summary of the Invention:

...USA 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B. B. [1983] in "Thioredoxins...

Description of the Invention:

...7% (w/w) ammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) **polyoxyethylene** (23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. All dilutions...in -20[degree(s)] C. in 0.05M Tris, pH 7.4 with 1 mM **EDTA** .

...with 0.1 M Tris, pH 7.5, containing 0.5M NaCl and 1 mM **EDTA** . The column was washed with two column volumes of the equilibrating buffer containing 2M urea...

...cm column of Sephadex(TM) G-25-40 equilibrated with 0.05M Tris, 1 mM **EDTA** , pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280...

15/3,KWIC/29 (Item 28 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3045751
Derwent Accession: 1987-158828
Utility
EXPIRED
C/ **Thioredoxin shufflease and use thereof**
Inventor: Pigiet, Vincent P., Winchester, MA
Rusche, James R., Worcester, MA
Schuster, Barbara J., State College, PA
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Wiseman, Thomas G. (Art Unit: 185)
Assistant Examiner: Patterson, Jr., Charles L.
Combined Principal Attorneys: Saliwanchik, Roman; Saliwanchik, David R.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 4904602	A	19900227	US 86894421	19860808
CIP	Abandoned			US 85802569	19851127

Fulltext Word Count: 6059

Summary of the Invention:
...USA, 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B. B. [1983] in "Thioredoxins...YM10 filter (Amicon, Danvers, Mass.). The buffer was exchanged with 50 mM Tris, 3 mM **EDTA** , pH 7.4 by diluting and concentrating the sample. The sample was stored at 4...

...reduced enzyme in 0.1M Tris, pH 7.4 or 9.0 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease or thioredoxin. At various times aliquots were assayed and...by diluting the inactive RNase into 0.1M Tris, pH 7.4 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease and/or reduced DTT. At various times aliquots were ...

Description of the Invention:
...5 ml 6X SSC (1X SSC=0.15M NaCl, 0.015M sodium citrate, 1 mM **EDTA**) and 10X Denhardt's solution (100 X--2% bovine serum albumin, 2% ficoll, 2% polyvinyl...At pH 9.0 (0.1M Tris, 1.0 mM **EDTA**) thioredoxin shufflease or a mixture of thioredoxin shufflease and oxidized DTT increased the rate of...

...At pH 7.4 (0.1M Tris, 1 mM **EDTA**) thioredoxin shufflease significantly increased the rate of refolding as compared to air oxidation. The time... At pH 8.5 (0.1M Tris, 1.0 mM **EDTA**) thioredoxin shufflease increased the rate of reactivation of scrambled RNase as compared to air oxidation...

15/3,KWIC/30 (Item 29 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3034492
Derwent Accession: 1989-220454
Utility
EXPIRED
C/ **Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations**
; SYNERGISTIC MIXTURE WITH SULFITES AND BISULFITES APPLIED

Inventor: Pigiet, Vincent P., Neshanic Station, NJ
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Ore, Dale R. (Art Unit: 125)
Combined Principal Attorneys: Saliwanchik, David R.; Saliwanchik, Roman

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 4894223	A	19900116	US 88140353	19880104
CIP	Pending			US 85770498	19850828
CIP	Abandoned			US 84674893	19841126

Disclaimer Date: 20050419

Fulltext Word Count: 3257

Summary of the Invention:
...A. 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. [1983] in "Thioredoxins...

Description of the Invention:
...7% (w/w) ammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) **polyoxyethylene** (23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. All dilutions...in -20[degree(s)] C. in 0.5M Tris, pH 7.4 with 1 mM **EDTA** .
...

...equilibrated with 0.1M Tris, pH 7.5, containing 0.5M NaCl and 1 mM **EDTA** . The column was washed with two column volumes of the equilibrating buffer containing 2M urea...

...cm column of Sephadex(TM) G-25-40 equilibrated with 0.05M Tris, 1 mM **EDTA** , pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280

15/3,KWIC/31 (Item 30 from file: 654)
DIALOG(R)File 654:US Pat.Full.

2902171
Derwent Accession: 1987-258442
Utility
EXPIRED
C/ **Method and ophthalmic composition for the prevention and reversal of cataracts**
; TOPICAL ADMINISTRATION OF THIOREDOXIN
Inventor: Pigiet, Vincent P., Winchester, MA
Spector, Abraham, New York, NY
Assignee: Trustees of Columbia University in the city of New York(02), New York, NY
COLUMBIA UNIVERSITY (Code: 08871)
Examiner: Brown, J. R. (Art Unit: 183)
Assistant Examiner: Moezie, F. T.
Combined Principal Attorneys: Saliwanchik, Roman; Saliwanchik, David R.; White, John P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 4771036	A	19880913	US 86828112	19860210

Fulltext Word Count: 3845

Summary of the Invention:
...USA, 75, 5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B. B. [1983] in "Thioredoxins...

Description of the Invention:
...25 mM KCl, 10 mM NaCl, 1.1 mM MgCl[sub]2, 0.1 mM **ethylenediaminetetraacetic** acid (**EDTA**), 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) pH 7.2 (buffer A...

15/3,KWIC/32 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00851452
PRODUCTION AND USE OF PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENECITY
VARIANTS DE PROTEINES A IMMUNOGENICITE MODIFIEE
Patent Applicant/Assignee:
NOVOZYMES A S, Krogshojvej 36, DK-2880 Bagsvaerd, DK, DK (Residence), DK (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
ROGGEN Erwin Ludo, Asavaenget 14, DK-2800 Lyngby, DK, DK (Residence), BE (Nationality), (Designated only for: US)
ERNST Steffen, Edelsmindevej 18, DK-2700 Bronshoj, DK, DK (Residence), DK (Nationality), (Designated only for: US)
SVENDSEN Allan, Overdamsvej 13, DK-2970 Horsholm, DK, DK (Residence), DK (Nationality), (Designated only for: US)
FRIIS Esben Peter, Langagervej 15, 2. tv., DK-2500 Valby, DK, DK (Residence), DK (Nationality), (Designated only for: US)
VON DER OSTEN Claus, Christian Winthers Vej 15, DK-2800 Lyngby, DK, DK (Residence), DK (Nationality), (Designated only for: US)
Legal Representative:
NOVOZYMES A S (commercial rep.), Krogshojvej 36, DK-2880 Bagsvaerd, DK,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200183559 A2-A3 20011108 (WO 0183559)
Application: WO 2001DK293 20010430 (PCT/WO DK0100293)
Priority Application: DK 2000707 20000428; US 2000203345 20000510; DK 2001327 20010228; US 2001277817 20010321
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ
TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 146189

Fulltext Availability:
Detailed Description
Claims
Detailed Description
... binding properties to three monoclonal antibod(inverted exclamation mark)es by immobilizing the peptides on **polyethylene** pins and bind30 ing a dilution series of each antibody to the pins. This reference...

Claim
... be coated by methods known

in the art. Examples of waxy coating materials are poly(**ethylene** oxide) products (**polyethylene** 91ycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 lo to 50 **ethylene** oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 **ethylene** oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acidsplexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), **ethylene** diaminetetraacetic acid (**EDTA**), **diethylenetriaminepentaacetic** acid (DTMPA) , alky1- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6...

...detergent may comprise one or more polymers. Examples are carboxymethy1cellulose (CMC), poly(vinylpyrrolidone) (PVP), poly **ethyleneglycol** (PEG), poly(viny1 alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and. laury1...

...or percarbonate which may be com~
bined with a peracid-forming bleach activator such as **tetraacetylethylenediamine** (TAED) or nonanoyloxybenzenesulfon~ate (NOBS).
3o Alternatively, the bleaching system may comprise peroxyacids of, e...Defensin HNP-1 (human neutrophil peptide) HNP-2 and HNP-3; bDefensin-12, Drosomycin, 91- **purothionin** , and Insect defensin A. Examples of b-sheet, peptides are Lactoferricin B, Tachyplesin I, and...DMG-buffer)
- Sodium Borate, borax (Sigma)
- 3,3-Dimethy1 glutaric acid (Sigma)
- Tween 20: Poly **oxyethylene** sorbitan mono laurate (Merck cat no. 822184)
s - PMSF (pheny1 methy1 sulfony1 flouride) from Sigma...

15/3,KWIC/33 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00840858
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES CANCEREUSES
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY, Office of Technology Transfer, 1020 Locust Street, Room M6, Philadelphia, PA, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
WALDMAN Scott A, 119 Bleddyn Road, Ardmore, PA 19003, US, US (Residence), US (Nationality), (Designated only for: US)
PARK Jason, 925 Latimer Street, Philadelphia, PA 19107, US, US (Residence), US (Nationality), (Designated only for: US)
SCHULZ Stephanie, 117 Howard Road, West Chester, PA 19380, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DeLUCA Mark (agent), Woodcock Washburn Kuritz Mackiewicz & Norris LLP, One Liberty Place, 46th floor, Philadelphia, PA 19103, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200173133 A1 20011004 (WO 0173133)
Application: WO 2001US9918 20010327 (PCT/WO US0109918)
Priority Application: US 2000192229 20000327
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 37104

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorabicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudoinonas exotoxin, diphtheria toxin, Clostridiunz... methotrexate, doxorabicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridiunz...dextrose, fatty,oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.
The vaccines of the present...were synthesized. Complementary oligonucleotides in 1 0 mM Tris-HCI (pH 7.5), 1 mM **EDTA** were annealed in a Hybaid Thermal Cycler by a programmed ramp in temp from 95...

Claim
... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/34 (Item 3 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00840857
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS OF ALIMENTARY CANAL ORIGIN
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES CANCEREUSES PROVENANT DU TUBE DIGESTIF
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY, Office of Technology Transfer, 1020 Locust Street, Room M6, Philadelphia, PA, US, US (Residence), US (Nationality) , (For all designated states except: US)
Patent Applicant/Inventor:
WALDMAN Scott A, 119 Bleddyn Road, Ardmore, PA 19003, US, US (Residence), US (Nationality), (Designated only for: US)
PARK Jason, 925 Latimer Street, Philadelphia, PA 19107, US, US (Residence), US (Nationality), (Designated only for: US)
SCHULZ Stephanie, 117 Howard Road, West Chester, PA 19380, US, US

(Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DELUCA Mark (agent), Woodcock Washburn Kurtz Mackiewicz & Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200173132 A1 20011004 (WO 0173132)
Application: WO 2001US9790 20010327 (PCT/WO US0109790)
Priority Application: US 2000192229 20000327
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 27589

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

- 34 Toxins are useful as...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens... fatty oils of vegetable 62 origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present...

Claim
... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, treninion, ricin, 76 ricin A chain, Pseudomonas exotoxin, diphtheria toxin...

15/3,KWIC/35 (Item 4 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00830501
MEMBRANE ESTROGEN RECEPTOR-DIRECTED THERAPY IN BREAST CANCER
THERAPIE DIRIGEE SUR LE RECEPTEUR MEMBRANAIRE DES OESTROGENES, DANS LE CANCER DU SEIN
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 1111 Franklin Street, 12th

00754251
X-RAY GUIDED DRUG DELIVERY
ADMINISTRATION DE MEDICAMENT GUIDEE PAR RAYON X
Patent Applicant/Assignee:
VANDERBILT UNIVERSITY, Office of Technology Transfer, Suite 210, 1207 17th Avenue South, Nashville, TN 37212, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
HALLAHAN Dennis E, 4214 Estes Road, Nashville, TN 37215, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
TAYLOR Arles A Jr, Jenkins & Wilson, P.A., University Tower, Suite 1400, 3100 Tower Boulevard, Durham, NC 27707, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 200066182 A1 20001109 (WO 0066182)
Application: WO 2000US11485 20000428 (PCT/WO US0011485)
Priority Application: US 99302456 19990429
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU CA JP US
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Filing Language: English
Fulltext Word Count: 41390

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... I m e t h a c ry I a t e,
polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, **polyethyleneglycol** , and polyaspartamide. In a preferred embodiment, the hydrophilic polymer is **polyethyleneglycol** (PEG), preferably as a PEG chain
having a molecular weight between 500-10,000 daltons...

...sodium lactate, sodium
phosphate, Tris, and N-methyl glucamine. Examples of suitable chelating agents include **EDTA** , DTPA, DTPA-BMA and salts and complexes thereof especially calcium, sodium or meglumine salts, e. g. edetate disodium, edetic acid, calcium **EDTA** .
Examples of suitable anti-oxidants include ascorbic acid, ascorbyl palmitate, cysteine, monothioglycerol, butylated hydroxyanisole, butylated...

...which aid in the Iyophilization and reconstitution
processes include sodium chloride, sorbitol, mannitol, glucose and **polyethyleneglycol** .

Representative starting materials and method for the preparation of
1 0 liposomes are also disclosed...

...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macrornomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin...of endothelial cell cultures was verified by staining for factor
VIII. ConfluentcellswereharvestedwithO.1%collagenaseO.01% **EDTAand** subcultured at a ...these liposomes bind to lipophilic proteins in the

serum, which reduces the circulation time.
Therefore, **polyethyleneglycol** (PEG) is used to coat the drug delivery systems.

PEG prolongs circulation time (Nam, Scontaining 1 OmM **EDTA** and 0.08% NaN3is added 5x excess of freshly prepared Traut's reagent in the...

...liposomes. Prepared vesicles and thiolated protein is mixed in 10mm Hepes, 0.15M NaCl and **EDTA** pH 6 The finai concentrations for proteins and liposomes are 0.25 g/L and...

Claim
... 4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin, ...daunorubicin, cytosine arabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cyclophosphamide, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine...4 fluorouracil, melphalan, chlorambucil, a nitrogen mustard, cyclophosphamide, cis-platinum, vindesine, vinca alkaloids, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinonederivatives, trenimon, steroids, aminopterin, anthracyclines, demecolcine, etoposide, mithramycin, doxorubicin, daunomycin, vinblastine, neocarzinostatin...group consisting of polyvinylpyrrolidone, polyvinylmethylether, polymethyloxazoline, polyethyloxazoline, polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxymethylcellulose, hydroxyethylcellulose, **polyethyleneglycol** , polyaspartamide and combinations thereof.
146. The delivery vehicle of claim 142, wherein the targeting agent...

15/3,KWIC/38 (Item 7 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00746101
BARLEY GENE FOR THIOREDOXIN AND NADP-THIOREDOXIN REDUCTASE
GENE D'ORGE POUR REDUCTASE DE THIOREDOXINE ET DE THIOREDOXINE NADP
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 5th floor, 1111 Franklin Street, Oakland, CA 94607-5200, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
CHO Myeong-Je, 3419 Solomon Lane, Alameda, CA 94502, US, US (Residence), KR (Nationality), (Designated only for: US)
DEL VAL Greg, 6612 Schmidt Lane, #4, El Cerrito, CA 94530, US, US (Residence), CH (Nationality), (Designated only for: US)
CAILLAU Maxime, Notre-Dame de la Croix, F-82600 Verdun-sur-Garonne, FR, FR (Residence), FR (Nationality), (Designated only for: US)
LEMAUX Peggy G, 253 Corliss Drive, Moraga, CA 94556, US, US (Residence), US (Nationality), (Designated only for: US)
BUCHANAN Bob B, 19 Tamalpais Road, Berkeley, CA 94708, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DEHLINGER Peter J (agent), Iota Pi Law Group, 350 Cambridge Avenue, Ste. 250, Palo Alto, CA 94306-1546, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200058352 A2-A3 20001005 (WO 0058352)
Application: WO 2000US8566 20000331 (PCT/WO US0008566)
Priority Application: US 99127198 19990331; US 99169162 19991206; US

2000177740 20000121; US 2000177739 20000121
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 51098

Fulltext Availability:
Detailed Description

Detailed Description
... the BTRXh or NTR polypeptide to one of a variety of nonproteinaceous polymers, e.g., **polyethylene** glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos...may iF-,Iude, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; **polyethylene** glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment...4 w/v) of buffer (50 mM Tris-HCI buffer, pH 7.9, 1 mM **EDTA** , 0.5 mM PMSF (phenyimethysulfonyl fluoride), 2 mM e-amino-n caproic acid, 2 mM...temperature in 50 ml buffer (50 mM Tris-HC1 buffer, pH 7.91 1 mM **EDTA** , 0.5 mM PMSF, 2 mM e-amino-n caproic acid, 2 mM benzamidine-HCI...pg) and the following: 100 pmol potassiuirn phosphate buffer (pH 7.9). Ten pmol Na- **EDTA** ; 0.25 pmol NADPH; 0.2 pmol DTNB. The reaction is started by the addition...are then fused with an immortalized cell line using a suitable fusing agent, such as **polyethylene** glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986...1 1 Oven @Hybaid, Woodridge, NJ, USA) using a solution containing 6 x SSC, 10mM **EDTA** , 5X Ddnhardt's solution, 0.5% SDS and 1 00 ug/ml of boiled calf... or phosphate buffer, pH 7.8, 0.5 mM phenylmethyl sulfonyl fluoride [PIVISF], 1 mM **EDTA**) varied from 2 to 4 ml depending on the number of seeds used and the...

...4 w/v) of buffer [(50 mM Tris-HCI buffer, pH 7.99 1 mM **EDTA** , 0.5 mM PMSF (phenylmethysulfonyl fluoride)], 2 mM e-amino-n caproic acid, 2 mM ...and added to Tris-HCI buffer (50 mM, pH 7.9) supplemented with 1 mM **EDTA** and 0.5 mM PMSF (1:3 to 1:6, wt/vol ratio of tissue...Planta 171: 321 -331 Johnson TC, Wada K, Buchanan BB, Holmgren A (1987b) Reduction of **purothionin** by the 1 1 wheat seed thioredoxin system and potential function as a secondary thiol...

15/3,KWIC/39 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00745163 **Image available**
PLANTS TRANSFORMED WITH THIOREDOXIN
VALORISATION DE GRAINES ET DE SEMENCES TRANSFORMEES PAR THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 1111 Franklin Street 5th Floor, Oakland, CA 94607-5200, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
CHO Myeong-Je, 3419 Solomon Lane, Alameda, CA 94502, US, US (Residence), KR (Nationality), (Designated only for: US)
LEMAUX Peggy G, 253 Corliss Drive, Moraga, CA 94556, US, US (Residence), US (Nationality), (Designated only for: US)

BUCHANAN Bob B, 19 Tamalpais Road, Berkeley, CA 94708, US, US (Residence), US (Nationality), (Designated only for: US)
WONG Joshua, 9 Viewmont Terrace, San Francisco, CA 94080, US, US (Residence), US (Nationality), (Designated only for: US)
MARX Corina, 407 Sunny Slope, Oakland, CA 94610, US, US (Residence), UY (Nationality), (Designated only for: US)
Legal Representative:
OSMAN Richard Aron (agent), Science & Technology Law Group, 75 Denise Drive, Hillsborough, CA 94010, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200058453 A2-A3 20001005 (WO 0058453)
Application: WO 2000US8315 20000329 (PCT/WO US0008315)
Priority Application: US 99126736 19990329; US 99127198 19990331; US 99169162 19991206; US 2000177739 20000121; US 2000177740 20000121
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 34481

Fulltext Availability:
Detailed Description

Detailed Description
... methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; **polyethylene** glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment...or phosphate buffer, pH 7.81 0.5 mM phenylmethyl sulfonyl fluoride [PMSF], I mM **EDTA**) varied from 2 to 4 ml depending on the number of seeds used and the...4 w/v) of buffer [(50 mM Tris-HCI buffer, pH 7.91 1 mM **EDTA** , 0.5 mM PMSF (phenylmethysulfonyl fluoride)], 2 mM e-amino-n caproic acid, 2 mM...and added to Tris-HCI buffer (50 mM, pH 7.9) supplemented with 1 mM **EDTA** and 0.5 mM PMSF (1:3 to 1:6, wt/vol ratio of tissue...or phosphate buffer, pH 7.81 0.5 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM **EDTA**] varied from 2 to 4 ml depending on the number of seeds used and the...3-6 ml of 30 mM Tris-HCI, pH 7.9 buffer containing 1 mM **EDTA** and 1 mM mBBr is added and mixed for 1 min. After thawing the extract... cells. Planta 171:321
Johnson TC, Wada K, Buchanan BB, Holmgren A (1987b) Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

15/3,KWIC/40 (Item 9 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00731623 **Image available**
ALLEVIATION OF THE ALLERGENIC POTENTIAL OF AIRBORNE AND CONTACT ALLERGENS BY THIOREDOXIN
DIMINUTION DU POTENTIEL ALLERGENIQUE D'ALLERGENES PORTES PAR L'AIR OU AGISSANT PAR CONTACT A L'AIDE DE THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 2150 Shattuck Avenue, Suite 510, Berkeley, CA 94720-1620, US, US (Residence), US (Nationality)
Inventor(s):
BUCHANAN Bob B, 19 Tamalpais Road, Berkeley, CA 94708, US,

prior to 2004)
AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB
GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA
UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 226130
Fulltext Availability:
Detailed Description

Detailed Description
... Cell 11:1007 (1999) ., As another instance,,
it has been found that suppression of the **ethylene** forming
enzyme results in arrested ovule development and female
sterility that can be reversed by application of **ethylene** (D.

De Martinis et al. , Plant Cell 11: 1061 (1999)) . The ability
to manipulate fertility...signature
The following small plant proteins are evolutionary
related.

- Gamma-thionins from wheat endosperm (gamma
purothionins) and barley (gamma- hordothionins) which
are toxic to animal cells and inhibit protein
synthesis in...and well
described in the scientific and patent literature. The
introduction of DNA constructs using **polyethylene** glycol
precipitation is described in Paszkowski et al. EMBO J. 3:2717
(1984). Electroporation techniques...10 mM spermine 3.5 g Stabilize
chromatin and the
nuclear membrane
0.1 M **EDTA** 37.2 g **EDTA** inhibits nuclease
(disodium)
0.1 M Tris 12.1 g Buffer
0.8 M KC1...

...sarcosine (Sarkosyl) 20.0 g
0.1 M Tris 12.1 g
0.04 M **EDTA** (Disodium) 14.9 g
Adjust the pH to 9.5 after all +the components are...

...7. Add 15 ml, dropwise, cold 2% Sarkosyl, 0.1 M Tris, 0.04
M **EDTA** solution (pH 9.5) while swirling gently. This
lyses the nuclei. The solution will become...two days against several
changes (at least three times) of TE (10 mM Tris, 1mM
EDTA , pH 8) to remove the cesium chloride.

16. Remove the dialyzed DNA from the tubing...

...and load in 1% TPE-agarose gel (TPE is 90 mM Tris
phosphate,, 2 mM **EDTA** , pH 8) . If the lambda DNA in the
lambda control digests are completely digested, proceed...875 mM dTTP,
0.125 mM DIG dUTP)
TE buffer (10 mM Tris, 1 mM **EDTA** , pH 8)
Maleate buffer: In 700 ml of deionized distilled water,
dissolve 11.61 g...

...of the diluted control DNA in
dilution buffer (TE: 10 mM Tris and 1 mM **EDTA** , pH 8) as
shown in the following table.

DIG-labeled

control DNA Final Conc.

starting...is mixed with 0.9 ml of
protoplasts. The resulting suspension is mixed with 40%
polyethylene glycol (MW 8000, PEG 8000), by gentle inversion
a few times at room temperature for...

15/3,KWIC/42 (Item 11 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00517693 **Image available**
RECOMBINANT MAJOR ALLERGEN OF THE POLLEN OF ARTEMISIA VULGARIS (MUGWORT)
ALLERGENE PRINCIPAL RECOMBINE DU POLLEN D'i(ARTEMISIA VULGARIS) (ARMOISE)
Patent Applicant/Assignee:
BIOMAY PRODUKTIONS- UND HANDELSGESELLSCHAFT MBH,
FERREIRA Fatima,
RICHTER Klaus,
ENGEL Edwin,
EBNER Christof,
KRAFT Dietrich,
BREITENBACH Michael,
HIMLY Martin,
Inventor(s):
FERREIRA Fatima,
RICHTER Klaus,
ENGEL Edwin,
EBNER Christof,
KRAFT Dietrich,
BREITENBACH Michael,
HIMLY Martin,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9949045 A2 19990930
Application: WO 99AT81 19990325 (PCT/WO AT9900081)
Priority Application: AT 98539 19980326
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH
GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MD MG MK MN MW
MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE
DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR
NE SN TD TG
Publication Language: German
Fulltext Word Count: 3464

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... SSPE ist 0,18 M NaCI 0,01 M Natriumphosphat pH 7,4, Im M **EDTA**).
Ein erfindungsgemAsses Verfahren zur Herstellung eines Art vl Allergens ist durch die folgenden Schritte gekennzeichnet...synthetisiert und kOnnte eine Pollen-spezifische Funktion haben. Es zeigt Ahnlichkeit zur Familie der Gamma- **Purothionine** .

BEISPIEL.

Die Isolierung des fUr Art v la codierenden Klons wurde in folgender Weise durchgefUhrt...

Claim
... 0.1% SDS. ix SSPE = 0.18M NaCl, 0.OIM Natriumphosphat pH = 7.4, linm **EDTA**

eingesetzt werden.

12 Ein replikationsfAhiger prokaryotischer oder eukaryotischer Expressionsvektor, der DNA-MolekUle entsprechend den AnsprUchen...

15/3,KWIC/43 (Item 12 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00508857
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
JUNG Rudolf,
BEACH Larry R,
DRESS Virginia M,
RAO A Gururaj,
RANCH Jerome P,
ERTL David S,
HIGGINS Regina K,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9940209 A1 19990812
Application: WO 99US2061 19990127 (PCT/WO US9902061)
Priority Application: US 9820716 19980209
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 7355

Fulltext Availability:
Detailed Description

Detailed Description
... invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (I 976); incorporated herein in...the like, all in accordance with well-known procedures.

The introduction of DNA constructs using **polyethylene** glycol precipitation is described in Paszkowski et aL, Embo J. 3: 2717-2722 (1984). Electroporation...

15/3,KWIC/44 (Item 13 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00488770 **Image available**
INCREASING THE DIGESTIBILITY OF FOOD PROTEINS BY THIOREDOXIN REDUCTION
AMELIORATION DE LA DIGESTIBILITE DES PROTEINES ALIMENTAIRES PAR REDUCTION PAR LA THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
Inventor(s):
BUCHANAN Bob B,
DEL VAL Gregorio,
LOZANO Rosa M,

JIAO Jin-an,
WONG Joshua H,
YEE Boihon C,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9920122 A1 19990429
Application: WO 98US20662 19981001 (PCT/WO US9820662)
Priority Application: US 97953703 19971017
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 38007

Fulltext Availability:
Detailed Description

Detailed Description
... are soluble cereal seed proteins, rich in cystine. In the Johnson, et al. investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3.

(2) NADPH + Thioredoxin h -@i@NADP +
..X
Thioredoxin hrw
(3) **Purothionin** .,, + Thioredoxin -4,d --> Purothionirl.'d
+ Thioredoxin h
Cereal seeds such as wheat, rye, barley, corn...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** a-1 and 0 from durum wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** ce sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** a-1 and 0 samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried out in 100 mM potassium phosphate buffer, pH 7. 1, containing 10 MM **EDTA** and 16 % glycerol in a final volume of 0. 1 ml. As indicated, 0. 7
...extraplastidic proteins, this test has proved useful in several studies. A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et al. (1981), FEBS
...

...and DSG-2) were found to be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I).

The a-amylase...2 0
Ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
" **Purothionin** -al 6 4 1 39

30 ** **Purothionin** -0 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...same as
for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** a, 20 /ig or CM-1, 20 ttg was used). The results thus confirmed
the...fluorescent band migrating behind thioredoxin.

EXAMPLE 5
Thioredoxin-linked Reduction of
Other Trypsin Inhibitors and **Purothionins**
In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin-reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a,
failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981). FEBS Lett. 124:237-240). However, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and from durum wheat) detectably activated NADP-MDH (Table I). The two durum wheat **purothionins** also differed in their ability to activate FBPase.

The activity differences between these **purothionins** were unexpected in view of the strong similarity in their amino acid sequences (Jones, B...

...to undergo reduction
by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the a-type) by the SDS-PAGE fluorescence procedure.
EXAMPLE 6
Ouantitation of Reduction...

...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTR, 0.01; **purothionin** -0, 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...

...difference, other conditions
were as in Examples 1
% Reduction After
Protein 20 min 120 min
Purothionin -0 15 32
DSG- ...CM-1 a-arnylase inhibitors (147
and 210%, respectively); corn kernel trypsin inhibitor (424%); and **purothionin** (82, 133, and 120% for the ce, cel and 0 forms, respectively).

Glutaredoxin was ineffective...

...and ovornucoid
inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants...0. 1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing
10 mM **EDTA** at 30'C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0...

...mg/ml, 0.02 mg/ml, and 0.25 mM,
respectively. With DTT as reductant, **EDTA** and components of the NADP/ thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HO, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of
binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the
protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...methotrexate, doxcrubicin, dauncrubicin, cytosinarabinoside,
etoposide, 5-4 fluorouracil, melphalan, chlorambucil, Cis platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/47 (Item 16 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00416399
PEPTIDE WITH INHIBITORY ACTIVITY TOWARDS PLANT PATHOGENIC FUNGI
PEPTIDE POSSEDANT UNE ACTION INHIBITRICE A L'ENCONTRE DE CHAMPIGNONS PATHOGENES DE PLANTES
Patent Applicant/Assignee:
NOVARTIS AG,
VERNOOIJ Barnardus Theodorus Maria,
CLARE Debra Arwood,
CHANDLER Danielle Brost,
KRAMER Catherine Mae,
Inventor(s):
VERNOOIJ Barnardus Theodorus Maria,
CLARE Debra Arwood,
CHANDLER Danielle Brost,
KRAMER Catherine Mae,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9806860 A1 19980219
Application: WO 97EP4438 19970813 (PCT/WO EP9704438)
Priority Application: US 9623940 19960814
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 20033

Fulltext Availability:
Detailed Description

Detailed Description
... were pipetted onto these disks. Test solutions were Jactoferrin (10 microgram) lactoferricin B (10 microgram), **purothionin** (the positive control, at 0, 5t 10 and 20 microgram; purchased from Calbiochern) and buffer...

...5 days, fungal growth was clearly visible on the plates, except around the filterdiscs containing **purothionin** and lactoferricin B. The sizes of the inhibition zones are shown in 1. In the...
...microgram lactoferricin B showed a zone of inhibition of 4.95 mm,

whereas 20 microgram **purothionin** showed an inhibition zone of 4.5 mm. In the C. graminicola experiment, lactoferricin B produced a zone of inhibition of 1.7 mm, whereas **purothionin** produced an inhibition zone of 2.1 mm. Lactoterrin did not cause inhibition of fungal...

...25
Table 1: Inhibition of spore germination by lactoferricin B
zone of inhibition
Funaus Buffer **Purothionin** Lactoterricin B
20gg logg
0. maydis 0 mm 4.5 mm 4.95 mm
C...was added (0. 1 M LiClj 1 00 mM Tris pH 80 1 0 mM **EDTA** , 1% SIDS), followed by e qual volumes of water-saturated phenol and chloroform.

The RNA...

15/3,KWIC/48 (Item 17 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00394280 **Image available**
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
JUNG Rudolf,
HASTINGS Craig,
COUGHLAN Sean,
HU David,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9735023 A2 19970925
Application: WO 97US4409 19970319 (PCT/WO US9704409)
Priority Application: US 96618911 19960320
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN GH KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 15668

Fulltext Availability:
Detailed Description

Detailed Description
... invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can, J. Biochem.; Vol. 22; p.

83J; (1976); incorporated herein in its...pH 5.2 and concentrated in the dialysis bags to about 100 ml with dry **polyethyleneglycol** (PEG 8000), Precipitated contaminating globulin proteins are removed by centrifugation at 6000 Xg for 15...

15/3,KWIC/49 (Item 18 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00359094
PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES

probe. Oriented multibilayers were prepared by squeezing...of buffer (10 mM
Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM **EDTA**), mixing with a Vortex mixer for 15 min. Multilamellar dispersions were incubated in a helium...

15/3,KWIC/50 (Item 19 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00330288
NEUTRALIZATION OF FOOF ALLERGENS BY THIOREDOXIN
NEUTRALISATION D'ALLERGENES ALIMENTAIRES PAR LA THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
Inventor(s):
BUCHANAN Bob B,
KOBREHEL Karoly,
YEE Boihon C,
LOZANO Rosa,
FRICK Oscar L,
ERMEL Richard W,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9612799 A1 19960502
Application: WO 95US13206 19951018 (PCT/WO US9513206)
Priority Application: US 94326976 19941021
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 32222

Fulltext Availability:
Detailed Description

Detailed Description
... are soluble cereal seed proteins, rich in cystine. In the Johnson, et al, investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs, 2 and 3,
NTR,
(2) NADPH + Thioredoxin @ - NADP +
L&OX
Thioredoxin
(3) **Purothionin** ., + Thioredoxin hrw -+ **Purothioninrd** + Thioredoxin
Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice contain four...measures are taken to minimize shock, renal
failure and respitory failure, Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp,
Other vroteins
Purothionin a from bread wheat and **purothionins** a-1 and fl from durum wheat were kind gifts from Drs, D.D, Kasarda and B.L, Jones, respectively. The **purothionin** a sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrophoresis. The

purothionin a-1 and 0 samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried out in 100 mM potassium phosphate buffer, pH 7,1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0.1 ml. As indicated, 0,7...extraplastidic proteins, this test has proved useful in several studies, A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K, et al. (1981), FEBS...

...and DSG-2) were found to be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I). The a-amylase...2 0
ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
** **Purothionin** -al 6 4 1 39
** **Purothionin** @P 6 ...same as for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** a, 20 Ag or CM-1, 20 Ag was used), The results thus confirmed the...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a, failed to activate NADP-MDH (Table I) (Wada, K., et al, (1981), FEBS Lett. 124:237-240), However, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and a from durum wheat) detectably activated NADP-MDH (Table I), The two durum wheat **purothionins** also differed in their ability to activate FBPase, The activity differences between these **purothionins** were unexpected in view of the strong similarity in their amino acid sequences (Jones, B...

...undergo reduction by thioredoxin, A requirement for thioredoxin was observed for the reduction of -39@
purothionin (here the a@type) by the SDS-PAGE fluorescence procedure.

EXAMPLE 6
Ouantitation of Reduction...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTRJV 0.01; **purothionin** fl, 1*7; DSG-1j 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...

...difference, other conditions were as in Examples 1
Reduction After
Protein 20 min 120 min
Purothionin -0 15 32
DSG-1 2 2 38
Corn kernel trypsin
inhibitor 3 15
Bowman...

...CM-1
a@amylase inhibitors (147 and 210%, respectively); corn kernel trypsin inhibitor (424%); and **purothionin**

(82, 133, and 120% for the a, al and # forms, respectively). Glutaredoxin was ineffective in...

...and ovomucoid inhibitor), Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-amylase 5 inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 300C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0.024 mg/mlt OeO2 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/ thioredoxin system were omitted. Following reduction, aliquots ...HR (30 mM Tris-HCI, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography, Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...30 mM Tris-HCl, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** , Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

15/3,KWIC/51 (Item 20 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00326460
COMPOSITIONS AND METHODS FOR THE ABROGATION OF CELLULAR PROLIFERATION UTILIZING THE HUMAN IMMUNODEFICIENCY VIRUS Vpr PROTEIN
COMPOSITIONS ET PROCEDES PERMETTANT D'INTERROMPRE UNE PROLIFERATION CELLULAIRE A L'AIDE DE LA PROTEINE Vpr DU VIH
Patent Applicant/Assignee:
THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
APOLLON INC,
WEINER David B,
LEVY David N,
REFAELI Yosef,
WILLIAMS William V,
AYYAROO Velpandi,
Inventor(s):
WEINER David B,
LEVY David N,
REFAELI Yosef,
WILLIAMS William V,
AYYAROO Velpandi,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9608970 A1 19960328
Application: WO 95US12344 19950921 (PCT/WO US9512344)
Priority Application: US 94309644 19940921
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 19863

Fulltext Availability:
Detailed Description

benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens... methotrexate, doxcrubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 114 benzoquinone derivatives, trenimcn, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens...any material capable of binding proteins.

Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** tetraacetic acid (**EDTA**) . One skilled in the art would readily recognize other fluorescence-emitting metals as well as...Compound 2-D13 comprises bleomycin conjugated to SEQ ID NO:2.

Compound 2-D14 comprises **purothionin** con3ugated to SEQ ID NO:2.

Compound 2-D15 comprises macromomycin conjugated to SEQ ID...4 hours at room temperature in 0.4 Tris-HCl, pH 8.0 and 1mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...receptor binding activity of the ST peptide.

"'In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The "'In-DTPA is converted to...

Claim

... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 114 benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens...

15/3,KWIC/54 (Item 23 from file: 349)

00286605

ANTIMICROBIAL PROTEINS

PROTEINES ANTIMICROBIENNES

Patent Applicant/Assignee:
ZENECA LIMITED,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
REES Sarah Bronwen,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
REES Sarah Bronwen,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9504754 A1 19950216
Application: WO 94GB1636 19940729 (PCT/WO GB9401636)
Priority Application: GB 9316158 19930804; GB 9317816 19930827
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR BY CA CN CZ FI GE HU JP KE KG KP KR KZ LK LT LV MD MG MN MW NO NZ PL RO RU SD SI SK TJ TT UA US UZ VN AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Fulltext Word Count: 11516

Fulltext Availability:
Detailed Description

Detailed Description
... extraction buffer
containing 10 mM NaH2PO4, 15 mM Na 2HPO4, 100 mm
KC1, 2 mM **EDTA** and 2 mM thiourea. After
extraction, the slurry was mixed in a WARING
blender and...sample
buffer contained 200 mM Tris-HC1 (pH 8.3), 1% (w/v)
SDS, mM **EDTA** , 0.005% bromophenol blue and, unless
otherwise ...from Amaranthus
caudatus, seeds (Broekaert et al, 1992,
Biochemistry, 31: 4308-4314) and of ss- **purothionin**
from wheat endosperm (another type of plant seed
protein with antimicrobial activity; Redman DG and...

...sensitive to
the presence of all tested cations, the activities
of Ace-AMP1 and ss- **purothionin** seem to be rather
cation-stimulated although not by Cat+. The
antagonistic effect of Ca2...200
(nd = not determined)
TABLE 3
Antifungal activity of Ace-AMP1, Ac-AMP1 and
ss- **purothionin** on Fusarium culmorum in synthetic
medium supplemented with different cations
IC50 (gg/ml)
SMF +50...

...2 6
Ac-AMP1 4 100 100 50 rt;200 rt;200 rt;200
ss- **purothionin** 4 2 3 2 2 2.5 35
EXAMPLE 8
Anti-bacterial and anti-yeast...

15/3,KWIC/55 (Item 24 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00286604
COMPOSITIONS OF FUSION PROTEINS CONTAINING METALLOTHIONEIN AND TARGETING-PROTEIN STRUCTURAL COMPONENTS
COMPOSITIONS DE PROTEINES DE FUSION CONTENANT DES COMPOSANTS STRUCTURAUX DE METALLOTHIONEINE ET DE PROTEINE DE CIBLAGE
Patent Applicant/Assignee:
UNIVERSITY OF NEW MEXICO,
Inventor(s):
ZAMORA Paul,
GRIFFITH Jeffery K,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9504753 A1 19950216
Application: WO 94US8689 19940804 (PCT/WO US9408689)
Priority Application: US 93104628 19930811
Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Fulltext Word Count: 9406

Fulltext Availability:
Detailed Description

Detailed Description
... chelating groups that have been used or can be so used include polydentate carboxylic acids (**EDTA** , DPTA, and the ....polydentate polyamines (amino oxime), chelates containing both amide and sulfur groups [N,N'-bis(mercaptoacetamino) **ethylenediamine** ; diaminodithiol], and chelates containing carboxyl and sulfur groups (dimercaptosuccinic acid). The chelator may be labeled...the thionins, which are present in monocots as well as in dicots. Cereal seeds contain **purothionins** which are toxic to animals and have thioredoxin activity. This invention contemplates the use of...molecules. MT-TPA molecules are extracted from cellular pastes in an extraction buffer containing Tris, **EDTA** , lysozyme, and deoxycholate and the inclusion bodies collected by centrifugation. The inclusion bodies are then...with a variety of radiometal complexing agents such as glucoheptate, citric acid, tartrate, tartrate/phthalate, **ethylene** diamine tetraacetic acid, or other stabilizing and/or complexing agents known to those skilled in...

15/3,KWIC/56 (Item 25 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00267908 **Image available**
HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN
DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
RAO A Gururaj,
BEACH Larry R,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9416078 A2-A3 19940721
Application: WO 94US382 19940112 (PCT/WO US9400382)
Priority Application: US 933885 19930113
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AT AU BB BG BR BY CA CH CN CZ DE DK ES FI GB HU JP KP KR KZ LK LU LV MG MN MW NL NO NZ PL PT RO RU SD SE SK UA UZ VN AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 5778
Fulltext Availability:
Detailed Description

Detailed Description
... crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information.

Three-dimensional modeling of the protein...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

15/3,KWIC/57 (Item 26 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00234016
USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS
UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES

Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
BUCHANAN Bob B,
KOBREHEL Karoly,
YEE Boihon C,
WONG Joshua H,
LOZANO Rosa,
JIAO Jin-an,
SHIN Sungho,
Inventor(s):
BUCHANAN Bob B,
KOBREHEL Karoly,
YEE Boihon C,
WONG Joshua H,
LOZANO Rosa,
JIAO Jin-an,
SHIN Sungho,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9308274 A1 19930429
Application: WO 92US8595 19921008 (PCT/WO US9208595)
Priority Application: US 91109 19911012; US 922 19920825
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO PL RO RU SD SE US US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG
Publication Language: English
Fulltext Word Count: 33674

Fulltext Availability:
Detailed Description

Detailed Description
... are
soluble cereal seed proteins, rich in cystine, In the Johnson, et al, investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3,
(2) NADPH + Thioredoxin h NTR I NADP + Thioredoxin h,,
@d
(3) **Purothioninox** + Thioredoxin hd -+ **Purothionin** ,, + Thioredoxin hox
Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice contain...measures are taken to minimize shock, renal failure and respitory failure, Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...the E, coli NADP/Thioredoxin System.

Figs 5 is a graph showing the effect of **purothionin** a and CM-1 a-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the...PAGE (Coomassie Blue stain)
but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** a-1 and from durum wheat were kind gifts from Drs. D, D, Kasarda and B.L. Jones, respectively, The purothionim a sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrephoresis.

The **purothionin** a-1 and fl samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried out in
100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0, 1 ml, As indicated, 0,7...extraplastidic proteins, this test has proved useful in several studies. A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et ale (1981), FEBS...

...DSG-2) were f ound
to be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I), The a-amylase...ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
3 0 Thionins
** **Purothionin** -al 6 4 1 39
** **Purothionin** -P 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...

...5, conditions
were as in Fig. 4 except that the DSG proteins were omitted and **purothionin** ajr 20 Ag or CM-I,, 20 Ag was used) . The results thus confirmed the...thioredoxin in Fig, 7)e
EXAMPLE 5
Thioredoxin-linked Reduction of
Other Trypsin Inhibitors and **Purothionins**
In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...a thioredoxin requirement for reduction (data not shown) .

In confirmation of earlier results, thioredoxin@reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a , failed to activate NADP MDH (Table I) (Wada,, K.,, et al, (1981) , FEBS Lett, 124:237-240) . However,, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and 16 from duruin wheat) detectably activated NADP-MDH (Table I) , The two' durum wheat **purothionins** also differed in their ability to activate FBPase. The activity differences between these **purothionins** were unexpected in view of the strong similarity in their amino acid sequences (Jones . B...

...to undergo reduction by thioredoxin. A
requirement for thioredoxin was- observed for the reduction of **purothionin** (here the a-type) by the SDS PAGE fluorescence procedure (Fig, 7),
EXAMPLE 6
Ouantitation...following concentrations of proteins were used (nmoles) : thioredoxin., 0 a 08; NTRI 0 e 01; **purothionin** @fl, T,
le7; DSG-Ir 0.7; corn kernel trypsin inhibitor, 1.0;

Rs-AFP2 2 2 3 2
Rs-nsLTP 30 60 >1000 >1000
P- **purothionin** 4 3 le5 4
Mj-AMP2 2 2 25 20
increased by about 7-fold...AFP2,,
nor Rs-nsLTP affected cell viability after 24 h of incubation. In-contrast, 0- **purothionin** administered at 50 pg/ml decreased the viability of both cell types by more than...

Claim
... pI322.
A process of combating fungi or bacteria which comprises exposure to SIm2, y-l- **purothionin** , or another v.-amylase inhibitor protein.
An extraction process for producing a protein as claimed...

15/3,KWIC/59 (Item 28 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00230335
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Patent Applicant/Assignee:
IMPERIAL CHEMICAL INDUSTRIES PLC,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
TERRAS Franky Raymond Gerard,
VANDERLEYDEN Jozef,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
TERRAS Franky Raymond Gerard,
VANDERLEYDEN Jozef,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9304586 A1 19930318
Application: WO 92GB1574 19920828 (PCT/WO GB9201574)
Priority Application: GB 9118730 19910902
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG
Publication Language: English
Fulltext Word Count: 9050

Fulltext Availability:
Detailed Description

Detailed Description
... buffer containing-10 mM
NaH2 PO 4" 15 mK Na2HP041 100 mM KClf 2 mM **EDTAr** 2 mM thiourea and I mM PMSF. The homogenate was squeezed through cheesecloth and clarified...proteins contained 200 mm Tris-HCl (pH 8-3), 1% (w/v) SDS, 1 mM **EDTA** , 0*005% bromophenol bluer and the sample buffer for analysis of reduced proteins contained a...and napin sequences.
EXAMPLE 3
Isolation of the trypsin inhibitors from

barley seeds and cc- **purothionins** from wheat seeds.

It is known from the literature that barley (Hordeum vulgare L.) contains...N-terminal amino acid sequences of the trypsin inhibitors from barley and from WG11.

a- **Purothionin** was isolated from wheat (Triticum aestivum) seed flour essentially as described by Redman and Fisher...

...a linear gradient (40 min) from 0 to 40% acetonitrile in 0.1% TFA. The **purothionineluted** was two :incompletely resolved peaks at 34 and 35% acetonitrile, respectively, which represent the ml...

...Jones, 1977, Cereal Chem, 54, 511-523). Both isoforms were pooled, to yield the a- **purothionin** preparation.,
EXAMPLE 4
Anti-fungal activity assay.

Antifungal activity was measured by microspectrophotometry as previously...5
Antifungal activity of the radish and rapeseed 2S albumins, barley trypsin inhibitors and a- **purothionin** .

The antifungal potency of the radish 2S albumins, rapeseed 2S albumins, barley trypsin inhibitors and a- **purothionin** was assessed by the microspectrophotometric assay described in Example 4. Growth of fungi, collection and...

...1
ANTIFUNGAL ACTIVITY OF 2S ALBUMINS FROM RAPESEED AND RADISHe BARLEY I INHIBITORS AND a- **PUROTHIONIN**
Protein IC 50 (pg/ml)
Medium A Mediuin B
Fc Ab Ap Vd Fc Ab...
...65 >1000 >1000 >1000 >101
bti2 90 40 90 50 >1000 >1000 >1000 >101
a- **purothionin** 4 4 8 2 11 11 12
In medium A (low ionic strength), the 2S albumins from rapeseed and radish, the barley trypsin inhibitors btil and bti2 and a.- **purothionin** were active on all four fungi tested. The five isoform fractions of the radish 2S...inhibitors were completely inactive at concentrations up to 1 mg/ml. In contrast the a- **purothionin** remained active in medium B, although its specific activity was decreased by about two- to...

...30-fold on T hamatum,
EXAMPLE 7
Synergism between 2S albumins, barley trypsin inhibitor and =@- **purothionin** .

The synergistic antifungal effect of combinations of 2S albumins and a- **purothionin** , on the one hand, and barley trypsin inhibitors and m- **purothionin** , on the other hand, was measured as follows.

To serial dilutions of m- **purothionin** , a constant subinhibitory concentration of 2S albumins

or barley trypsin inhibitors was added. The IC so value of the a- **purothionin** was calculated from dose-response curves for the series with and without addition of 2S 3
SYNERGISTIC ANTIFUNGAL EFFECT OF COMBINATIONS BETWEEN 2S ALBUMINS/a@ **PUROTHIONIN** AND TRYPSIN INHIBITORS/=- **PUROTHIONIN**
Fungus Test IC 50 of a- **purothionin** in #g/ml
Protein (Synergism Factor)
Medium A Medium B
Test protein Test protein
conc...

...The highest synergism factor (73) was obtained in medium B for the combination of a.- **purothionin** and Bn-2S3 (at 250 jig/ml) on the fungus Alternaria brassicola. The synergism factors...

...2S albumins
Rs-2S3 and Bn-2S3.

EXAMPLE 8
Effect of 2S albumins and a- **purothionin** on bacterial growth.

Antibacterial activity was measured micro5pectrophotometrically as follows. A bacterial suspension was prepared...these bacteria.

Synergisms in antibacterial activity were assessed for combinations between Rs-2S3 and a.- **purothionin** by using the same approach as described in Example 7. A synergistic effect was observed...

...of 10, 50 and 250 jig/ml, respectively, to a serial dilution series of a- **purothionin** (Table 4). Thus, the thionin-potentiating activity of 2S albumin is not limited to fungi...
...TABLE 4
SYNERGISTIC ANTIBACTERIAL EFFECT ON BACILLUS MEGATERIUM OF COMBINATIONS BETWEEN RS-2S3 AND a- **PUROTHIONIN**
Rs-2S3 conc. IC so of a- **purothionin** in #g/ml (/Jg/ml) (Synergism Factor)
Medium C Medium D
2 2*5
0...

...Oe6(4)
Oo2(15)
250 Oe15(17)
EXAMPLE 9
Effect of 2S albumins and w- **purothionin** on cultured human cells.

Human cell toxicity assays were performed either on umbilical vein endothelial...Rs-2S3 did not affect cell viability after 24 hours of incubation. in contrast, cc- **purothionin** administered at 50 jig/ml and 20 jig/ml decreased the viability of both cell...
...a constant concentration of 250 ug/ml to a serial dilution series of a (x- **purothionin** did not increase the toxic activity of the-a- **purothionin** .

EXAMPLE 10

Main Patent	US 5942663	A	19990824	US 97915142	19970820
Division	US 5482928	A		US 93117080	19931220
Continuation	US 5689048	A		US 95471329	19950602
Priority				GB 915052	19910311
				GB 915684	19910319
				WO 92GB423	19920310

Fulltext Word Count: 6005

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The ...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...
...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as... time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. Also, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (.circle-solid...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/14 (Item 13 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4163914
Derwent Accession: 1995-246394
Utility
C/ **Transformed plants expressing antimicrobial proteins ; ANTIMICROBIAL PROTEIN CAPABLE OF ISOLATION FROM SEEDS OF HEUCHERA OR AESCULUS**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Osborn, Rupert William, Middlesex, GB
Rees, Sarah Bronwen, Bracknell, GB
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Robinson, Douglas W. (Art Unit: 169)
Assistant Examiner: Zaghmout, Ousama M-Faiz
Combined Principal Attorneys: Hohenschutz, Liza D.

Publication	Application	Filing

	Number	Kind	Date	Number	Date
Main Patent	US 5919918	A	19990706	US 97956459	19971023
Division	Pending			US 656318	
Priority				GB 9326424	19931224

Fulltext Word Count: 5637

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

Description of the Invention:
...sub]4, 15 mm Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/15 (Item 14 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4118486
Derwent Accession: 1995-178646
Utility
CERTIFICATE OF CORRECTION
C/ **Methods of treating metastatic colorectal cancer with ST receptor binding compounds**
; **RADIOACTIVE THERAPEUTIC AGENT, RECEPTOR BINDING MOIETY**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Green, Lora M. (Art Unit: 168)
Assistant Examiner: Ricigliano, Joseph W.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5879656	A	19990309	US 96583447	19960105
CIP	US 5518888	A	19960521	US 93141892	19931026

Fulltext Word Count: 33695

Description of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...Compound

Derwent Accession: 1994-183512
Utility
C/ **Transgenic plants expressing biocidal proteins**
; **ISOLATED ANTIMICROBIAL PROTEIN HAVING AMINO ACID SEQUENCECONTAINING COMMON CYSTEINE/GLYCINE DOMAIN OF CHITIN BINDINGPLANT PROTEINS; AGICULTURAL AND PHARMACEUTICAL APPLICATIONS AS FUNGICIDES OR BACETRICIDES**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Phillippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Forest Park, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Kemmerer, Elizabeth (Art Unit: 166)
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5986176	A	19991116	US 96777113	19961230
Division	US 5691199	A		US 95451566	19950526
Division	US 5514779	A		US 93149839	19931110
CIP	Abandoned			US 932842	19930114
CIP	Pending			WO 92GB999	19920603
Priority				GB 9112300	19910607
				GB 9223798	19921112
				GB 933564	19930223

Fulltext Word Count: 13574

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/13 (Item 12 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4187951
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; **TRANSFORMED BIOLOGICAL PROTEIN**
Inventor: De Bolle, Miguel, Leuven, BE
Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 169)
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date

polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens

15/3,KWIC/10 (Item 9 from file: 654)

4317418
Derwent Accession: 1995-178646
Utility
C/ **Compositions that specifically bind to colorectal cancer cells and methods of using the same**
; CONJUGATED COMPOUNDS WHICH COMPRISE AN ST RECEPTOR BINDING MOIETY AND A RADIOSTABLE ACTIVE MOIETY
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Brusca, John S. (Art Unit: 165)
Assistant Examiner: Larson, Thomas G.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6060037	A	20000509	US 96635930	19960426
CIP	US 5518888	A		US 93141892	19931026
PCT	WO 9511694		19950504	WO 94US12232	19941026
			371:19960426		
			102e:19960426		
	US 5601990	A		US 94305056	19940913

Fulltext Word Count: 39301

Description of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1, ... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin,

diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well asCompound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2...at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid) . DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/11 (Item 10 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4239629
Derwent Accession: 1994-249225
Utility
REISSUE REQUESTED **See File 123 for details
C/ **High lysine derivatives of [alpha]-hordothionin ; FOR TRANSFORMED PLANTS AND CELLS WITH ENHANCED LYSINE CONTENT AND WHICH SUPPRESS AND KILL PLANT PATHOGENS INCLUDING FUNGI**
Inventor: Rao, A. Gururaj, Urbandale, IA
Beach, Larry, Des Moines, IA
Assignee: Pioneer Hi-Bred International, Inc.(02), Des Moines, IA
Pioneer Hi-Bred International Inc (Code: 17947)
Examiner: Smith, Lynette R. F. (Art Unit: 169)
Assistant Examiner: Kimball, Melissa L.
Law Firm: Pioneer Hi-Bred International, Inc.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5990389	A	19991123	US 97838763	19970410
Continuation	Abandoned			US 95575654	19951220
Continuation	Abandoned			US 95369975	19950106
Continuation	Abandoned			US 933885	19930113

Fulltext Word Count: 4619

Description of the Invention:
...crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

15/3,KWIC/12 (Item 11 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4235015

4547046
Derwent Accession: 1995-178646
Utility
CERTIFICATE OF CORRECTION
C/ **Imaging of colorectal cancer using ST receptor binding compounds**
; VISUALIZING TUMOR CELLS; ADMINISTER RADIOACTIVE PARTICLES TO HUMANS AND DETECT POSITIONING AND ACCUMULATION OF RADIOACTIVE PARTICLES IN BODY
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Celsa, Bennett (Art Unit: 168)
Assistant Examiner: Ricigliano, Joseph W.
Law Firm: Woodcock Washburn Kurt Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6268159	A	20010731	US 98138237	19980821
Division	Pending			US 95468449	19950606
Division	US 5518888	A		US 93141892	19931026

Fulltext Word Count: 28331

Summary of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:
...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2... at room temperature in 0.4 M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]111 In-DTPA is ...

15/3,KWIC/6 (Item 5 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4458415 **IMAGE Available
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; AMINO ACID SEQUENCES OF MICROBIOCIDAL PROTEINS; FOR TRANSGENIC PLANTS WITH INCREASED DISEASE RESISTANCE
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE

Osborn, Rupert W., Middlesex, GB
Rees, Sarah B., Berkshire, GB
Terras, Franky R. G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: ZENECA Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Low, Christopher S. F. (Art Unit: 163)
Assistant Examiner: Gupta, Anish
Combined Principal Attorneys: Hohenschutz, Liza D.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6187904	A	20010213	US 97971982	19971117
Division	US 5538525	A		US 95377687	19950125
Continuation	US 5689043	A		US 95452078	19950526
Continuation	Abandoned			US 932480	19930104
Continuation	Abandoned			WO 92GB1570	19920827
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 11346

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored ...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**
(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml

15/3,KWIC/7 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4458048
Derwent Accession: 1998-456873
Utility

Rupert Osborn, INV
Sarah Rees, INV
Franky Terras, INV
Jozef Vanderleyden, INV
Assignee: ZENECA Limited(03)
Correspondence Address: SYNGENTA CROP PROTECTION, INC., 2 RIGHTER PARKWAY P.O. BOX 15458, WILMINGTON, DE, 19850-5458, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20010014732	A1	20010816	US 2001759584	20010112
Division	US 5538525			US 95377687	19950125
Continuation	US 6187904			US 97971982	19971117
Continuation	US 5689043			US 95452078	19950526
Continuation	ABANDONED			US 932480	19930104
Continuation	UNKNOWN			WO 92GB1570	19920827
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 14428

Summary of the Invention:
...Such proteins, including SI[small alpha, Greek]2 from sorghum and [small gamma, Greek]-1- **purothionin** from wheat, are known to inhibit insect [small alpha, Greek]-amylase and are toxic to...

Description of the Drawings:
...Lc-AFP, Ct-AMP1, sorghum SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...2PO[sub]4, 15 mM Na[sub]2HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [small gamma, Greek]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...small alpha, Greek]-amylase inhibitor SI[small alpha, Greek]2, wheat [small gamma, Greek]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...For the purpose of comparison, these tests were performed in parallel with [small beta, Greek]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...with both test fungi. In comparison, the IC[sub]50 value of [small beta, Greek]- **purothionin**
(...Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [small beta, Greek]- **purothionin** administered at 50 [small mu, Greek]g/ml decreased the viability of both cell types...

Exemplary or Independent Claim(s):
...or bacteria which comprises exposure to SI[small alpha, Greek]2, [small gamma, Greek]-1- **purothionin** , or another [small alpha, Greek]-amylase inhibitor protein.

15/3,KWIC/5 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; **ANTIFUNGAL, ISOLATED FROM PLANT SEEDS**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P.A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R.G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: McElwain, Elizabeth F. (Art Unit: 169)
Combined Principal Attorneys: Thomson, Marian T.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5824869	A	19981020	US 96777192	19961227
Division	US 5689043	A		US 95452078	19950526
Division	US 5538525	A		US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 11956

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed ...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in ...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/18 (Item 17 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3992551
Derwent Accession: 1996-188141
Utility
C/ **Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocoticoid receptor type II and viral protein R interacting protein**
; **DETECTING THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I PROTEIN**
Inventor: Weiner, David B., Merion, PA
Refaeli, Yosef, Boston, MA
Assignee: The Trustees of the University of Pennsylvania(02), Philadelphia, PA
Pennsylvania, University of (Code: 64664)
Examiner: Adams, Donald E. (Art Unit: 183)
Assistant Examiner: Parkin, Jeffrey S.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5763190	A	19980609	US 94309644	19940921

Fulltext Word Count: 15683

Summary of the Invention:
...capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified cellulose, polyacrylamide, agarose, and magnetite. The nature of the...metals can be attached to the TNF-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediaminetetraacetic** acid (**EDTA**).
...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:
...inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 [mu]g/ml; PMSF, 1 mM, and **EDTA** , 1 mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and...were then centrifuged at 10,000 G. These supernatants were supplemented with protease inhibitors (PMSF, **EDTA** , EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...HEPES (pH 7.9), 4 mM Tris (pH 7.9), 60 mM KCI, 1 mM **EDTA** , and 1 mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh...polyacrylamide gel (270 [mu]l 1M Tris, pH 7.9; 80 [mu]l 0.5M **EDTA** , pH 7.9; 13.2 [mu]l 1M sodium acetate, pH 7.9; 5.33...

...7.9; 13.2 ml 1M sodium Acetate, pH 7.9; 8 ml 0.5M **EDTA** , pH 8.0; up to a final volume of 4 liters with ddH2O). Gels were...

15/3,KWIC/19 (Item 18 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3979152
Derwent Accession: 1995-246394
Utility
C/ **Antimicrobial proteins**
; **PLANT EXTRACTS**
Inventor: Broekaert, Willem Frans, Dilbeek, BE

Cammue, Bruno Philippe Angelo, Alsemberg, BE
Osborn, Rupert William, Twickenham, GB
Rees, Sarah Bronwen, Forest Park, GB
Assignee: Zeneca Agrochemicals(03), GB, England
Zeneca Agrochemicals GB (Code: 45474)
Examiner: Lebuyader, John (Art Unit: 189)
Assistant Examiner: Wang, Andrew
Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5750504	A	19980512	US 96656318	19960612
PCT	WO 9518229		19950706	WO 94GB2766	19941219
			371:19960612		
			102e:19960612		

Fulltext Word Count: 5652

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including Si[alpha]2 from sorghum and g-1- **purothionin** (g-1P) from wheat, are known to inhibit insect [alpha]-amylase and may be toxic...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Two hundred...

15/3,KWIC/20 (Item 19 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3913150
Derwent Accession: 1994-183512
Utility
C/ **DNA encoding biocidal proteins**
; **DNA SEQUENCES IN PROTEINS AND VECTORS IN CELLS**
Inventor: Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Berkshire, GB England
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Hendricks, Keith D. (Art Unit: 184)
Law Firm: Cushman Darby & Cushman Intellectual Property Group of Pillsbury Madison & Sutro LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5691199	A	19971125	US 95451566	19950526
Division	US 5514779	A		US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 14086

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM

thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5, 5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/21 (Item 20 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3910716 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
; **GENETIC ENGINEERING**
Inventor: De Bolle, Miguel, Louvain, BE
Broekaert, Willem Frans, Dilbeek, BE
Cammue, Bruno Philippe Angelo, Alsemberg, BE
Rees, Sarah Bronwen, Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)
Law Firm: Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5689048	A	19971118	US 95471329	19950602
Division	US 5482928	A		US 93117080	19931220
Priority				GB 915052	19910311
				GB 915684	19910319

Fulltext Word Count: 6067

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in..,

...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (.circle-solid...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/22 (Item 21 from file: 654)

3910711
Derwent Accession: 1993-100978
Utility
C/ **Biocidal proteins**
; **GENETIC ENGINEERING**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P.A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R.G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)
Assistant Examiner: McElwain, Elizabeth F.
Law Firm: Cushman Darby & Cushman Intellectual Property Group of Pillsbury Madison & Sutro, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5689043	A	19971118	US 95452078	19950526
Division	US 5538525	A		US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829
				GB 923038	19920213
				GB 9213526	19920625

Fulltext Word Count: 11738

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM

thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively. For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/23 (Item 22 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3811293
Derwent Accession: 1994-183512
Utility
C/ **Biocidal proteins**
; **BACTERICIDES, FUNGICIDES**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Forest Park, GB England
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Hendricks, Keith D. (Art Unit: 184)
Law Firm: Cushman Darby & Cushman, L.L.P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5597801	A	19970128	US 95451568	19950526
Division	Pending			US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 14080

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/24 (Item 23 from file: 654)

3746704
Derwent Accession: 1993-100978
Utility
CM/ **Biocidal proteins**
; **ANTIFUNGAL OR ANTIBACTERIAL AGENTS**

Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Osborn, Rupert W., Middlesex, GB England
Rees, Sarah B., Berkshire, GB England
Terras, Franky R. G., Amzegem, BE
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Fox, David T. (Art Unit: 183)
Assistant Examiner: McElwain, Elizabeth F.
Law Firm: Cushman Darby & Cushman, L.L.P.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5538525	A	19960723	US 95377687	19950125
Continuation	Abandoned			US 932480	19930104
Priority				GB 9118523	19910829

Fulltext Word Count: 11737

Summary of the Invention:
...Biochem, 194:533-539). Such proteins, including SI[alpha]2 from sorghum and [gamma]-1- **purothionin** from wheat, are known to inhibit insect [alpha]-amylase and are toxic to insect larvae...

Description of the Drawings:
...AMP1, the Cb-AMPs, Lc-AFP, Ct-AMP1, sorghum SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted products of the pea genes pI230 and pI39, of the cowpea gene...

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, and 1 mM PMSF. The homogenate was squeezed through cheesecloth and clarified...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...Sorghum bicolor(Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to [gamma]- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett, 270, 191-194) which inhibit in...

...Lc-AFP, Ct-AMP1, the sorghum [alpha]-amylase inhibitor SI[alpha]2, wheat [gamma]1 **purothionin** , and the predicted sequences of the mature protein products of the Fusarium-induced pea genes...supplement, respectively.

For the purpose of comparison, these tests were performed in parallel with [beta]- **purothionin** , an antifungal protein from wheat seeds (isolated as described in Redman and Fisher, 1969, J...30-fold with both test fungi. In comparison, the IC[sub]50 value of [beta]- **purothionin**

(...AFP2, nor Rs-nsLTP affected cell viability after 24 h of incubation. In contrast, [beta]- **purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/25 (Item 24 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3725121
Derwent Accession: 1995-178646
Utility
C/ **ST receptor binding compounds and methods of using the same ; IMAGING METASTASIZED COLORECTAL CANCER, TOXTIN PEPTIDES LESS THAN 25 UNITS**
Inventor: Waldman, Scott A., Ardmore, PA
Assignee: Thomas Jefferson University(02), Philadelphia, PA
Jefferson, Thomas University (Code: 06943)
Examiner: Scheiner, Toni R. (Art Unit: 182)
Assistant Examiner: Green, Lora M.
Law Firm: Woodcock Washburn Kurtz Mackiewicz & Norris

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5518888	A	19960521	US 93141892	19931026

Fulltext Word Count: 28768
Summary of the Invention:
...g. cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

Description of the Invention:
...Compound 2-D14 comprises **purothionin** conjugated to SEQ ID NO:2... hours at room temperature in 0.4M Tris-HCl, pH 8.0 and 1 mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...activity of the ST peptide. [sup]111 In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The [sup]113 In-DTPA is ...

15/3,KWIC/26 (Item 25 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3720578
Derwent Accession: 1994-183512
Utility
C/ **Biocidal proteins from plants**
; **CHITINASES, FUNGICIDES**
Inventor: Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Forest Park, GB England
Vanderleyden, Jozef, Heverlee, BE
Assignee: Zeneca Limited(03), London, GB, England
Zeneca Ltd GB (Code: 32757)
Examiner: Wax, Robert A. (Art Unit: 184)
Assistant Examiner: Hendricks, Keith D.
Law Firm: Cushman Darby & Cushman

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 5514779	A	19960507	US 93149839	19931110
CIP	Abandoned			US 932842	19930114
Priority				GB 9112300	19910607
				GB 9223708	19921112
				GB 933564	19930223

Fulltext Word Count: 13401

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...

...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithioerythritol (DTE). Proteins were...

...in 6M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and 1 mM **EDTA** . The mixtures were allowed to react with 5,5'-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, [beta]-**purothionin** administered at 50 [mu]g/ml decreased the viability of both cell types by more...

15/3,KWIC/27 (Item 26 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3685299 **IMAGE Available
Derwent Accession: 1992-331736
Utility
C/ **Biocidal proteins**
Inventor: De Bolle, Miguel, Leuven, BE
Broekaert, Willem F., Dilbeek, BE
Cammue, Bruno P. A., Alsemberg, BE
Rees, Sarah B., Bracknell, GB
Vanderleyden, Jozef, Heverlee, BE
Assignee: Imperial Chemical Industries PLC(03), London, GB, England
Imperial Chemical Industries Ltd GB (Code: 41248)
Examiner: Furman, Keith C. (Art Unit: 184)
Law Firm: Cushman, Darby & Cushman

	Publication Number	Kind	Date	Application Number	Filing Date

Main Patent	US 5482928	A	19960109	US 93117080	19931220
PCT	WO 9215691		19920917	WO 92GB423	19920310
		371:	19931220		
		102e:	19931220		
Priority				GB 915052	19910311
				GB 915684	19910319

Fulltext Word Count: 6028

Description of the Invention:
...sub]4, 15 mM Na[sub]2 HPO[sub]4, 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed... buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...agglutinin or UDA (Broekaert, W. F. et al; 1989; Science, 245, 1100-1102) and [beta]- **purothionin** (Hernandez-Lucas, C. et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...previously described (Peumans, W. J. et al; 1983; FEBS Lett, 177, 99-103). The [beta]- **purothionin** was purified from wheat endosperm by the method of Redman, D. G. and Fisher, N...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and [beta]- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...
...g/ml for Mj-AMP2, from 0.5 to 15 [mu]g/ml for [beta]- **purothionin** , and from 20 to over 1,000 [mu]g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2=[beta]- **purothionin** >Mj-AMP1>UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to [beta]- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and [beta]- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and [beta]- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. FIG. 4 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and [beta]- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (. . .), after 60...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. [beta]- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/28 (Item 27 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3181895
Derwent Accession: 1989-220454
Utility
EXPIRED
C/ **Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations**
Inventor: Pigiet, Vincent P., Neshanic Station, NJ
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Page, Thurman K. (Art Unit: 152)
Assistant Examiner: Rucker, Susan S.
Law Firm: Saliwanchik & Saliwanchik

Publication Number	Kind	Date	Application Number	Filing Date

Main Patent	US 5028419	A	19910702	US 89397802	19890823
Division	US 4894223	A		US 88140353	19880104
CIP	US 4919924	A		US 85770498	19850828
CIP	Abandoned			US 84674893	19841126

Disclaimer Date: 20070424

Fulltext Word Count: 3722

Summary of the Invention:
...USA 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B. B. [1983] in "Thioredoxins...

Description of the Invention:
...7% (w/w) ammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) **polyoxyethylene** (23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. All dilutions...in -20[degree(s)] C. in 0.05M Tris, pH 7.4 with 1 mM **EDTA** .
...with 0.1 M Tris, pH 7.5, containing 0.5M NaCl and 1 mM **EDTA** . The column was washed with two column volumes of the equilibrating buffer containing 2M urea...

...cm column of Sephadex(TM) G-25-40 equilibrated with 0.05M Tris, 1 mM **EDTA** , pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280...

15/3,KWIC/29 (Item 28 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3045751
Derwent Accession: 1987-158828
Utility
EXPIRED
C/ **Thioredoxin shufflease and use thereof**
Inventor: Pigiet, Vincent P., Winchester, MA
Rusche, James R., Worcester, MA
Schuster, Barbara J., State College, PA
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Wiseman, Thomas G. (Art Unit: 185)
Assistant Examiner: Patterson, Jr., Charles L.
Combined Principal Attorneys: Saliwanchik, Roman; Saliwanchik, David R.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 4904602	A	19900227	US 86894421	19860808
CIP	Abandoned			US 85802569	19851127

Fulltext Word Count: 6059

Summary of the Invention:
...USA, 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B. B. [1983] in "Thioredoxins...YM10 filter (Amicon, Danvers, Mass.). The buffer was exchanged with 50 mM Tris, 3 mM **EDTA** , pH 7.4 by diluting and concentrating the sample. The sample was stored at 4...

...reduced enzyme in 0.1M Tris, pH 7.4 or 9.0 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease or thioredoxin. At various times aliquots were assayed and...by diluting the inactive RNase into 0.1M Tris, pH 7.4 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease and/or reduced DTT. At various times aliquots were

...

Description of the Invention:
...5 ml 6X SSC (1X SSC=0.15M NaCl, 0.015M sodium citrate, 1 mM **EDTA**) and 10X Denhardt's solution (100 X--2% bovine serum albumin, 2% ficoll, 2% polyvinyl...At pH 9.0 (0.1M Tris, 1.0 mM **EDTA**) thioredoxin shufflease or a mixture of thioredoxin shufflease and oxidized DTT increased the rate of...

...At pH 7.4 (0.1M Tris, 1 mM **EDTA**) thioredoxin shufflease significantly increased the rate of refolding as compared to air oxidation. The time... At pH 8.5 (0.1M Tris, 1.0 mM **EDTA**) thioredoxin shufflease increased the rate of reactivation of scrambled RNase as compared to air oxidation...

15/3,KWIC/30 (Item 29 from file: 654)
DIALOG(R)File 654:US Pat.Full.

3034492
Derwent Accession: 1989-220454
Utility
EXPIRED
C/ **Use of thioredoxin, thioredoxin-derived, or thioredoxin-like dithiol peptides in hair care preparations**
; **SYNERGISTIC MIXTURE WITH SULFITES AND BISULFITES APPLIED**
Inventor: Pigiet, Vincent P., Neshanic Station, NJ
Assignee: Repligen Corporation(02), Cambridge, MA
Repligen Corp (Code: 10790)
Examiner: Ore, Dale R. (Art Unit: 125)
Combined Principal Attorneys: Saliwanchik, David R.; Saliwanchik, Roman

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 4894223	A	19900116	US 88140353	19880104
CIP	Pending			US 85770498	19850828
CIP	Abandoned			US 84674893	19841126

Disclaimer Date: 20050419

Fulltext Word Count: 3257

Summary of the Invention:
...A. 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. [1983] in "Thioredoxins...

Description of the Invention:
...7% (w/w) ammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) **polyoxyethylene** (23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. All dilutions...in -20[degree(s)] C. in 0.5M Tris, pH 7.4 with 1 mM **EDTA** .
...

...equilibrated with 0.1M Tris, pH 7.5, containing 0.5M NaCl and 1 mM **EDTA** . The column was washed with two column volumes of the equilibrating buffer containing 2M urea...

...cm column of Sephadex(TM) G-25-40 equilibrated with 0.05M Tris, 1 mM **EDTA** , pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280

15/3,KWIC/31 (Item 30 from file: 654)
DIALOG(R)File 654:US Pat.Full.

2001327 20010228; US 2001277817 20010321
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ
TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 146189

Fulltext Availability:
Detailed Description
Claims
Detailed Description
... binding properties to three monoclonal antibod(inverted exclamation mark)es by immobilizing the peptides on **polyethylene** pins and bind30 ing a dilution series of each antibody to the pins. This reference...

Claim
... be coated by methods known
in the art. Examples of waxy coating materials are poly(**ethylene** oxide) products (**polyethylene** 91ycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 lo to 50 **ethylene** oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 **ethylene** oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acidsplexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), **ethylene** diaminetetraacetic acid (**EDTA**), **diethylenetriaminepentaacetic** acid (DTMPA) , alky1- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6...

...detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), poly **ethyleneglycol** (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and. laury1...

...or percarbonate which may be com~
bined with a peracid-forming bleach activator such as **tetraacetylethylenediamine** (TAED) or nonanoyloxybenzenesulfon~ate (NOBS).
3o Alternatively, the bleaching system may comprise peroxyacids of, e...Defensin HNP-1 (human neutrophil peptide) HNP-2 and HNP-3; bDefensin-12, Drosomycin, 91- **purothionin** , and Insect defensin A. Examples of b-sheet, peptides are Lactoferricin B, Tachyplesin I, and...DMG-buffer)
- Sodium Borate, borax (Sigma)
- 3,3-Dimethyl glutaric acid (Sigma)
- Tween 20: Poly **oxyethylene** sorbitan mono laurate (Merck cat no. 822184)
s - PMSF (phenyl methyl sulfonyl flouride) from Sigma...

15/3,KWIC/33 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00840858
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES

CANCEREUSES

Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY, Office of Technology Transfer, 1020 Locust Street, Room M6, Philadelphia, PA, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
WALDMAN Scott A, 119 Bleddyn Road, Ardmore, PA 19003, US, US (Residence), US (Nationality), (Designated only for: US)
PARK Jason, 925 Latimer Street, Philadelphia, PA 19107, US, US (Residence), US (Nationality), (Designated only for: US)
SCHULZ Stephanie, 117 Howard Road, West Chester, PA 19380, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DeLUCA Mark (agent), Woodcock Washburn Kuritz Mackiewicz & Norris LLP, One Liberty Place, 46th floor, Philadelphia, PA 19103, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200173133 A1 20011004 (WO 0173133)
Application: WO 2001US9918 20010327 (PCT/WO US0109918)
Priority Application: US 2000192229 20000327
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 37104

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorabicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudoinonas exotoxin, diphtheria toxin, Clostridiunz... methotrexate, doxorabicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridiunz...dextrose, fatty,oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.
The vaccines of the present...were synthesized. Complementary oligonucleotides in 1 0 mM Tris-HCI (pH 7.5), 1 mM **EDTA** were annealed in a Hybaid Thermal Cycler by a programmed ramp in temp from 95...

Claim

... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/34 (Item 3 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00840857
COMPOSITIONS AND METHODS FOR IDENTIFYING AND TARGETING CANCER CELLS OF ALIMENTARY CANAL ORIGIN
COMPOSITIONS ET PROCEDES D'IDENTIFICATION ET DE CIBLAGE DE CELLULES CANCEREUSES PROVENANT DU TUBE DIGESTIF
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY, Office of Technology Transfer, 1020 Locust Street, Room M6, Philadelphia, PA, US, US (Residence), US (Nationality) , (For all designated states except: US)
Patent Applicant/Inventor:
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PARK Jason, 925 Latimer Street, Philadelphia, PA 19107, US, US (Residence), US (Nationality), (Designated only for: US)
SCHULZ Stephanie, 117 Howard Road, West Chester, PA 19380, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DELUCA Mark (agent), Woodcock Washburn Kurtz Mackiewicz & Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200173132 A1 20011004 (WO 0173132)
Application: WO 2001US9790 20010327 (PCT/WO US0109790)
Priority Application: US 2000192229 20000327
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 27589

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

- 34 Toxins are useful as...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** ,

macromomycin, 1,4benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens... fatty oils of vegetable 62 origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present...

Claim
... methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, treninion, ricin, 76 ricin A chain, Pseudomonas exotoxin, diphtheria toxin...

15/3,KWIC/35 (Item 4 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00830501
MEMBRANE ESTROGEN RECEPTOR-DIRECTED THERAPY IN BREAST CANCER
THERAPIE DIRIGEE SUR LE RECEPTEUR MEMBRANAIRE DES OESTROGENES, DANS LE CANCER DU SEIN
Patent Applicant/Assignee:
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Patent Applicant/Inventor:
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MARQUEZ-GARBAN Diana C, 3464 Lisa Place, Sherman Oaks, CA 91403, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
WOOD William J (agent), Gates & Cooper, 6701 Center Drive West, Suite 1050, Los Angeles, CA 90045, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200162288 A1 20010830 (WO 0162288)
Application: WO 2001US5897 20010223 (PCT/WO US0105897)
Priority Application: US 2000185026 20000225
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 28454

Fulltext Availability:
Detailed Description

Detailed Description
... myeloma lines such as NS-1 or P3NS-1, in the presence of, e.g., **polyethylene** glycol.

The hybridomas or lymphoblastoid cells which secrete antibody of interest can be identified by...doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, n-dtomycin, bleomycin, **purothionin** , macromomycin, 1,4benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas

exotoxin, diphtheria toxin, 5 Clostridium...

15/3,KWIC/36 (Item 5 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00784061
NUCLEIC ACID SEQUENCES ENCODING CELL WALL-DEGRADING ENZYMES AND USE TO ENGINEER RESISTANCE TO FUSARIUM AND OTHER PATHOGENS
SEQUENCES D'ACIDE NUCLEIQUE CODANT POUR DES ENZYMES DEGRADANT LES PAROIS CELLULAIRES ET LEUR UTILISATION POUR CREER UNE RESISTANCE AU FUSARIUM ET A D'AUTRES PATHOGENES
Patent Applicant/Assignee:
THE UNITED STATES OF AMERICA as represented by THE SECRETARY OF AGRICULTURE, 1400 Independence Avenue SW, Washington, DC 20250-0302, US , US (Residence), US (Nationality)
NOVO NORDISK BIOTECH INC, 1445 Drew Avenue, Davis, CA 95616-4880, US, US (Residence), US (Nationality)
Inventor(s):
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HOHN Thomas M, 102 Blakely Drive, Chapel Hill, NC 27514, US,
BERKA Randy M, 3609 Modoc Place, Davis, CA 95616, US,
Legal Representative:
PENDORF Stephan A (et al) (agent), Pendorf & Cutliff, P.O. Box 20445, Tampa, FL 33622-0445, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200116353 A1 20010308 (WO 0116353)
Application: WO 2000US23802 20000830 (PCT/WO US0023802)
Priority Application: US 99151582 19990830; US 2000224946 20000811; US 2000649747 20000828
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 40894

Fulltext Availability:
Detailed Description
Detailed Description
... leaves were inhibitory to F. solani (Molina et al., 1993). A combination of a wheat **purothionin** and a 2S albumin from radish or oilseed rape was effective against the growth of...in 0.9 M NaCl, 0.09
M Tris-HCl pH 7.6, 6 mM **EDTA** , 0.5% NP-401 IX Denhardt's solution, 1 mM sodium pyrophosphate, 1 mM sodium...were partitioned on 1W agarose in 40 mM TRIS
acetate, pH 8.21 1 mM **EDTA** , stained with an ethidium bromide solution (@l pg/mL), and visualized by irradiation with an...containing 0.5 M
sucrose, 80 mM potassium chloride, 10 mM TRIS-chloride, 10 mM **EDTA** ,, 4 mM spermine, 1 mM spermidine, pH 9.51 180 mg/L phenylmethylsulfonyl fluoride (added ...ML
of a buffer solution containing 50 mM TRIS-chloride, pH 81 100 mM - 95
EDTA , 100 mM sodium chloride, and 600 @tg of proteinase K. An additional 4 mL of...

00746101

BARLEY GENE FOR THIOREDOXIN AND NADP-THIOREDOXIN REDUCTASE
GENE D'ORGE POUR REDUCTASE DE THIOREDOXINE ET DE THIOREDOXINE NADP

Patent Applicant/Assignee:
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DEL VAL Greg, 6612 Schmidt Lane, #4, El Cerrito, CA 94530, US, US (Residence), CH (Nationality), (Designated only for: US)
CAILLAU Maxime, Notre-Dame de la Croix, F-82600 Verdun-sur-Garonne, FR, FR (Residence), FR (Nationality), (Designated only for: US)
LEMAUX Peggy G, 253 Corliss Drive, Moraga, CA 94556, US, US (Residence), US (Nationality), (Designated only for: US)
BUCHANAN Bob B, 19 Tamalpais Road, Berkeley, CA 94708, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
DEHLINGER Peter J (agent), Iota Pi Law Group, 350 Cambridge Avenue, Ste. 250, Palo Alto, CA 94306-1546, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200058352 A2-A3 20001005 (WO 0058352)
Application: WO 2000US8566 20000331 (PCT/WO US0008566)
Priority Application: US 99127198 19990331; US 99169162 19991206; US 2000177740 20000121; US 2000177739 20000121
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 51098

Fulltext Availability:
Detailed Description

Detailed Description

... the BTRXh or NTR polypeptide to one of a variety of nonproteinaceous polymers, e.g., **polyethylene** glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos...may iF-,Iude, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; **polyethylene** glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment...4 w/v) of buffer (50 mM Tris-HCI buffer, pH 7.9, 1 mM **EDTA** , 0.5 mM PMSF (phenyimethysulfonyl fluoride), 2 mM e-amino-n caproic acid, 2 mM...temperature in 50 ml buffer (50 mM Tris-HC1 buffer, pH 7.91 1 mM **EDTA** , 0.5 mM PMSF, 2 mM e-amino-n caproic acid, 2 mM benzamidine-HCI...pg) and the following: 100 pmol potassiuirn phosphate buffer (pH 7.9). Ten pmol Na- **EDTA** ; 0.25 pmol NADPH; 0.2 pmol DTNB. The reaction is started by the addition...are then fused with an immortalized cell line using a suitable fusing agent, such as **polyethylene** glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986...1 1 Oven @Hybaid, Woodridge, NJ, USA) using a solution containing 6 x SSC, 10mM **EDTA** , 5X Ddnhardt's solution, 0.5% SDS and 1 00 ug/ml of boiled calf... or phosphate buffer, pH 7.8, 0.5 mM phenylmethyl sulfonyl fluoride [PIVISF], 1 mM **EDTA**) varied from 2 to 4 ml depending on the number of seeds used and the...

...4 w/v) of buffer [(50 mM Tris-HCI buffer, pH 7.99 1 mM **EDTA** , 0.5 mM PMSF (phenylmethysulfonyl fluoride)], 2 mM e-amino-n caproic acid, 2 mM ...and added to Tris-HCI buffer (50 mM, pH 7.9) supplemented with 1 mM **EDTA** and 0.5 mM PMSF (1:3 to 1:6, wt/vol ratio of tissue...Planta 171: 321 -331 Johnson TC, Wada K, Buchanan BB, Holmgren A (1987b) Reduction of **purothionin** by the 1 1 wheat seed thioredoxin system and potential function as a secondary thiol...

15/3,KWIC/39 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00745163 **Image available**
PLANTS TRANSFORMED WITH THIOREDOXIN
VALORISATION DE GRAINES ET DE SEMENCES TRANSFORMEES PAR THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 1111 Franklin Street 5th Floor, Oakland, CA 94607-5200, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
CHO Myeong-Je, 3419 Solomon Lane, Alameda, CA 94502, US, US (Residence), KR (Nationality), (Designated only for: US)
LEMAUX Peggy G, 253 Corliss Drive, Moraga, CA 94556, US, US (Residence), US (Nationality), (Designated only for: US)
BUCHANAN Bob B, 19 Tamalpais Road, Berkeley, CA 94708, US, US (Residence), US (Nationality), (Designated only for: US)
WONG Joshua, 9 Viewmont Terrace, San Francisco, CA 94080, US, US (Residence), US (Nationality), (Designated only for: US)
MARX Corina, 407 Sunny Slope, Oakland, CA 94610, US, US (Residence), UY (Nationality), (Designated only for: US)
Legal Representative:
OSMAN Richard Aron (agent), Science & Technology Law Group, 75 Denise Drive, Hillsborough, CA 94010, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200058453 A2-A3 20001005 (WO 0058453)
Application: WO 2000US8315 20000329 (PCT/WO US0008315)
Priority Application: US 99126736 19990329; US 99127198 19990331; US 99169162 19991206; US 2000177739 20000121; US 2000177740 20000121
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 34481

Fulltext Availability:
Detailed Description

Detailed Description
... methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; **polyethylene** glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment...or phosphate buffer, pH 7.81 0.5 mM phenylmethyl sulfonyl fluoride [PMSF], I mM **EDTA**) varied from 2 to 4 ml depending on the number of seeds used and the...4 w/v) of buffer [(50 mM Tris-HCI buffer, pH 7.91 1 mM **EDTA** , 0.5 mM PMSF (phenylmethysulfonyl fluoride)], 2 mM e-amino-n caproic acid,

2 mM...and added to Tris-HCI buffer (50 mM, pH 7.9) supplemented with 1 mM **EDTA** and 0.5 mM PMSF (1:3 to 1:6, wt/vol ratio of tissue...or phosphate buffer, pH 7.81 0.5 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM **EDTA**] varied from 2 to 4 ml depending on the number of seeds used and the...3-6 ml of 30 mM Tris-HCI, pH 7.9 buffer containing 1 mM **EDTA** and 1 mM mBBr is added and mixed for 1 min. After thawing the extract... cells. Planta 171:321
Johnson TC, Wada K, Buchanan BB, Holmgren A (1987b) Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

15/3,KWIC/40 (Item 9 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00731623 **Image available**
ALLEVIATION OF THE ALLERGENIC POTENTIAL OF AIRBORNE AND CONTACT ALLERGENS BY THIOREDOXIN
DIMINUTION DU POTENTIEL ALLERGENIQUE D'ALLERGENES PORTES PAR L'AIR OU AGISSANT PAR CONTACT A L'AIDE DE THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, 2150 Shattuck Avenue, Suite 510, Berkeley, CA 94720-1620, US, US (Residence), US (Nationality)
Inventor(s):
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DEL VAL Gregorio, 6612 Schmidt Lane, No. 4, El Cerrito, CA 94530, US,
LOZANO Rosa M, Olivos de Penanevada, 5-8degreesA, E-28937 Mostoles, ES,
WONG Joshua H, 9 Viewmont Terrace, South San Francisco, CA 94080, US,
YEE Boihon C, 3215 Primrose Lane, Walnut Creek, CA 94598, US,
FRICK Oscar L, 370 Parnassus Avenue, San Francisco, CA 94117, US,
Legal Representative:
SMITH Karen S (et al) (agent), Flehr Hohbach Test Albritton & Herbert LLP, 4 Embarcadero Center, Suite 3400, San Francisco, CA 94111-4187, US ,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200044781 A1 20000803 (WO 0044781)
Application: WO 2000US1958 20000125 (PCT/WO US0001958)
Priority Application: US 99238379 19990127
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 44560

Fulltext Availability:
Detailed Description

Detailed Description
... are soluble cereal seed proteins, rich in cystine. In the Johnson, et al. investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3.

(2) NADPH + Thioredoxin h @-@NADP +
M
Thioredoxin h,ed
(3) **Purothionin** ,,,, + Thioredoxin **Purothionin** ,,

,d
+ Thioredoxin ho,,
Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice ...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite
and excising the wound area, there are no known...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** ; a-1 and 0 from durum
wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** a sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** a-1 and 0 samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried
out in 100 mM potassium phosphate buffer, pH 7.1, containing 10 MM **EDTA** and 16 % glycerol in a final volume of 0. 1 ml. As indicated, 0.7 ...

...extraplastidic proteins, this test has proved useful in several studies. A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et al. (1 98...

...and DSG-2) were found to
be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I).

The a-amylase...2 0
Ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
" **Purothionin** -cil 6 4 1 39
** **Purothionin** -0 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...

...same as
for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** a, 20 ttg or CM-1, 20 ttg was used). The results thus confirmed
the...fluorescent band migrating behind thioredoxin.

EXAMPLE 5
Thioredoxin-linked Reduction of
Other Trypsin Inhibitors and **Purothionins**
In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...

...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin-reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a,
failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981), FEBS Lett. 124:237-240). However, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and from durum. wheat) detectably activated NADP-MDH (Table I). The two durum wheat Durothionins also differed in their abilitv to activate FBPase.

The activitv differences between these **purothiorins** were unexpected in view of the strong similarity in their amino acid sequences (Jones, B...

...to undergo reduction
by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the a-type) by the SDS-PAGE fluorescence procedure.

EXAMPLE 6
Quantitation of Reduction...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTR, 0.01; **purothionin** -0, 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...
...difference, other conditions
were as in Examples 1
% Reduction After
Protein 20 min 120 min
Purothionin -0 15 32
DSG-1 22 38
Corn kernel trypsin inhibitor 3 15
Bowman-Birk...

...CM-1 a-amylase inhibitors (147
and 210%, respectively); corn kernel trypsin inhibitor (424%); and **purothionin** (82, 133, and 120% for the a, al and 0 forms, respectively).

Glutaredoxin was ineffective...

...and ovornucoid
inhibitor). Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-arnylase inhibitors (DSG-1,
CM-1), a cystine-rich trypsin inhibitor from plants...0. 1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing
10 mM **EDTA** at 30'C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0and 0.25 mM,
respectively. With DTT as reductant, **EDTA** and components of the NADP/ thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCI, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...

...30 mM Tris
HO, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...in wheat flour is about 0.01 % (Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat seed thioredoxin system and potential function as a secondary thiol messenger in...

...mM Tris-HC1 pH 7.4 containing 1 liM phenylmethylsulfonyl
fluoride (PMSF) and 1 mM **EDTA** -Na] and stirred gently for 30 min at room temperature. The mixture was then centrifuged...

15/3,KWIC/41 (Item 10 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00577322
SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY
FRAGMENTS D'ADN DETERMINES SELON LEUR SEQUENCE ET POLYPEPTIDES

CORRESPONDANTS CODES PAR LESDITS FRAGMENTS

Patent Applicant/Assignee:
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Patent Applicant/Inventor:
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Legal Representative:
STEWART Raymond C (et al) (agent), Birch, Stewart, Kolasch & Birch, LLP, P.O. Box 747, Falls Church, VA 22040-0747, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200040695 A2-A3 20000713 (WO 0040695)
Application: WO 2000US466 20000107 (PCT/WO US0000466)
Priority Application: US 99115293 19990108
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 226130
Fulltext Availability:
Detailed Description

Detailed Description

... Cell 11:1007 (1999) ., As another instance,, it has been found that suppression of the **ethylene** forming enzyme results in arrested ovule development and female sterility that can be reversed by application of **ethylene** (D.

De Martinis et al. , Plant Cell 11: 1061 (1999)) . The ability to manipulate fertility...signature
The following small plant proteins are evolutionary related.

- Gamma-thionins from wheat endosperm (gamma **purothionins**) and barley (gamma- hordothionins) which are toxic to animal cells and inhibit protein synthesis in...and well described in the scientific and patent literature. The introduction of DNA constructs using **polyethylene** glycol precipitation is described in Paszkowski et al. EMBO J. 3:2717 (1984). Electroporation techniques...10 mM spermine 3.5 g Stabilize chromatin and the nuclear membrane
0.1 M **EDTA** 37.2 g **EDTA** inhibits nuclease (disodium)
0.1 M Tris 12.1 g Buffer
0.8 M KCl...

...sarcosine (Sarkosyl) 20.0 g
0.1 M Tris 12.1 g
0.04 M **EDTA** (Disodium) 14.9 g
Adjust the pH to 9.5 after all +the components are...

...7. Add 15 ml, dropwise, cold 2% Sarkosyl, 0.1 M Tris, 0.04 M **EDTA** solution (pH 9.5) while swirling gently. This lyses the nuclei. The solution will become...two days against several changes (at least three times) of TE (10 mM Tris, 1mM **EDTA** , pH 8) to remove the cesium chloride.

16. Remove the dialyzed DNA from the tubing...

...and load in 1% TPE-agarose gel (TPE is 90 mM Tris phosphate,, 2 mM **EDTA** , pH 8) . If the lambda DNA in the lambda control digests are completely digested, proceed...875 mM dTTP, 0.125 mM DIG dUTP)
TE buffer (10 mM Tris, 1 mM **EDTA** , pH 8)
Maleate buffer: In 700 ml of deionized distilled water, dissolve 11.61 g...

...of the diluted control DNA in dilution buffer (TE: 10 mM Tris and 1 mM **EDTA** , pH 8) as shown in the following table.

DIG-labeled
control DNA Final Conc.

starting...is mixed with 0.9 ml of protoplasts. The resulting suspension is mixed with 40% **polyethylene** glycol (MW 8000, PEG 8000), by gentle inversion a few times at room temperature for...

15/3,KWIC/42 (Item 11 from file: 349)

00517693 **Image available**
RECOMBINANT MAJOR ALLERGEN OF THE POLLEN OF ARTEMISIA VULGARIS (MUGWORT)
ALLERGENE PRINCIPAL RECOMBINE DU POLLEN D'i(ARTEMISIA VULGARIS) (ARMOISE)
Patent Applicant/Assignee:
BIOMAY PRODUKTIONS- UND HANDELSGESELLSCHAFT MBH,
FERREIRA Fatima,
RICHTER Klaus,
ENGEL Edwin,
EBNER Christof,
KRAFT Dietrich,
BREITENBACH Michael,
HIMLY Martin,
Inventor(s):
FERREIRA Fatima,
RICHTER Klaus,
ENGEL Edwin,
EBNER Christof,
KRAFT Dietrich,
BREITENBACH Michael,
HIMLY Martin,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9949045 A2 19990930
Application: WO 99AT81 19990325 (PCT/WO AT9900081)
Priority Application: AT 98539 19980326
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH
GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MD MG MK MN MW

MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE
DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR
NE SN TD TG
Publication Language: German
Fulltext Word Count: 3464

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... SSPE ist 0,18 M NaCI 0,01 M Natriumphosphat pH 7,4, Im M **EDTA**).
Ein erfindungsgemAsses Verfahren zur Herstellung eines Art vl Allergens ist durch die folgenden Schritte gekennzeichnet...synthetisiert und kOnnte eine Pollen-spezifische Funktion haben. Es zeigt Ahnlichkeit zur Familie der Gamma- **Purothionine** .

BEISPIEL.

Die Isolierung des fUr Art v la codierenden Klons wurde in folgender Weise durchgefUhrt...

Claim
... 0.1% SDS. ix SSPE = 0.18M NaC1, 0.OIM Natriumphosphat pH = 7.4, linm **EDTA**
eingesetzt werden.

12 Ein replikationsfAhiger prokaryotischer oder eukaryotischer Expressionsvektor, der DNA-MolekUle entsprechend den AnsprUchen...

15/3,KWIC/43 (Item 12 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00508857
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
JUNG Rudolf,
BEACH Larry R,
DRESS Virginia M,
RAO A Gururaj,
RANCH Jerome P,
ERTL David S,
HIGGINS Regina K,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9940209 A1 19990812
Application: WO 99US2061 19990127 (PCT/WO US9902061)
Priority Application: US 9820716 19980209
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM
HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW
MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES
FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN
TD TG
Publication Language: English
Fulltext Word Count: 7355

Fulltext Availability:
Detailed Description

Detailed Description
... invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (I 976); incorporated herein in...the like, all in accordance with well-known procedures.

The introduction of DNA constructs using **polyethylene** glycol precipitation is described in Paszkowski et aL, Embo J. 3: 2717-2722 (1984). Electroporation...

15/3,KWIC/44 (Item 13 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00488770 **Image available**
INCREASING THE DIGESTIBILITY OF FOOD PROTEINS BY THIOREDOXIN REDUCTION
AMELIORATION DE LA DIGESTIBILITE DES PROTEINES ALIMENTAIRES PAR REDUCTION PAR LA THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
Inventor(s):
BUCHANAN Bob B,
DEL VAL Gregorio,
LOZANO Rosa M,
JIAO Jin-an,
WONG Joshua H,
YEE Boihon C,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9920122 A1 19990429
Application: WO 98US20662 19981001 (PCT/WO US9820662)
Priority Application: US 97953703 19971017
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 38007

Fulltext Availability:
Detailed Description

Detailed Description
... are soluble cereal seed proteins, rich in cystine. In the Johnson, et al. investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3.

(2) NADPH + Thioredoxin h -@i@NADP +
..X
Thioredoxin hrw
(3) **Purothionin** .,, + Thioredoxin -4,d --> Purothionirl.'d
+ Thioredoxin h
Cereal seeds such as wheat, rye, barley, corn...measures are taken to minimize shock, renal failure and respitory failure. Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...PAGE (Coomassie Blue stain), but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** a-1 and 0 from durum

wheat were kind gifts from Drs. D.D. Kasarda and B.L. Jones, respectively. The **purothionin** ce sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrophoresis. The **purothionin** a-1 and 0 samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried
out in 100 mM potassium phosphate buffer, pH 7. 1, containing 10 MM **EDTA** and 16 % glycerol in a final volume of 0. 1 ml. As indicated, 0. 7
...extraplastidic proteins, this test has proved useful in several studies. A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et al. (1981), FEBS
...

...and DSG-2) were found to
be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I).

The a-amylase...2 0
Ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
" **Purothionin** -al 6 4 1 39
30 ** **Purothionin** -0 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...same as
for the DSG/DTNB assay except that the DSG proteins were omitted and **purothionin** a, 20 /ig or CM-1, 20 ttg was used). The results thus confirmed
the...fluorescent band migrating behind thioredoxin.

EXAMPLE 5
Thioredoxin-linked Reduction of
Other Trypsin Inhibitors and **Purothionins**
In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...a thioredoxin requirement for reduction (data not shown).

In confirmation of earlier results, thioredoxin-reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a,
failed to activate NADP-MDH (Table I) (Wada, K. et al. (1981). FEBS Lett. 124:237-240). However, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and from durum wheat) detectably activated NADP-MDH (Table I). The two durum wheat **purothionins** also differed in their ability to activate FBPase.

The activity differences between these **purothionins** were unexpected in view of the strong similarity in their amino acid sequences (Jones, B...

...to undergo reduction
by thioredoxin. A requirement for thioredoxin was observed for the reduction of **purothionin** (here the a-type) by the SDS-PAGE fluorescence procedure.
EXAMPLE 6
Ouantitation of Reduction...

...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTR, 0.01; **purothionin** -0, 1.7; DSG-1, 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...

...difference, other conditions

were as in Examples 1
% Reduction After
Protein 20 min 120 min
Purothionin -0 15 32
DSG- ...CM-1 a-arnylase inhibitors (147
and 210%, respectively); corn kernel trypsin inhibitor (424%); and
purothionin (82, 133, and 120% for the ce, cel and 0 forms,
respectively).

Glutaredoxin was ineffective...

...and ovornucoid
inhibitor). Those proteins that were reduced by either thioredoxin or
glutaredoxin include the **purothionins** , two a-amylase inhibitors (DSG-1,
CM-1), a cystine-rich trypsin inhibitor from plants...0. 1 ml of 20 mM
sodium phosphate buffer, pH 7.9 containing
10 mM **EDTA** at 30'C for 2 hours. The concentrations of thioredoxin,
NTR, and NADPH were 0...

...mg/ml, 0.02 mg/ml, and 0.25 mM,
respectively. With DTT as reductant, **EDTA** and components of the NADP/
thioredoxin system were omitted. Following reduction, aliquots of the
inhibitor...HR (30 mM Tris-HO, pH 7.5, containing 200 mM NaCl and 1 mM
EDTA) chromatography. Pullulanase inhibitor protein was purified as
described below.

CM32 Chromatography
The pullulanase inhibitor sample...30 mM Tris
HC1, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** . Fractions (3.6
ml/fraction) showing pullulanase inhibitory activity were pooled,
concentrated by dialysis against...in wheat flour is about 0.01 %
(Johnson, T.C. et al. (1987), "Reduction of **purothionin** by the wheat
seed thioredoxin system and potential function as a secondary thiol
messenger in...

15/3,KWIC/45 (Item 14 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00476374
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE LIENT SPECIFIQUEMENT AUX CELLULES CANCEREUSES COLORECTALES ET UTILISATION DE CES COMPOSITIONS
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
Inventor(s):
WALDMAN Scott A,
PEARLMAN Joshua M,
BARBER Michael T,
SCHULZ Stephanie,
PARKINSON Scott J,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9907726 A1 19990218
Application: WO 98US16440 19980807 (PCT/WO US9816440)
Priority Application: US 97908643 19970807
Designated States:
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AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 36129

Fulltext Availability:
Detailed Description

Detailed Description
... any material capable of binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite.

The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**).

One skilled in the art would readily recognize other fluorescence-emitting metals as well as...g.

cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium... ofmethotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and **polyethylene** glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present...mM sodium deoxycholate, 3.5 mM sodium dodecyl sulfate, 0.5 @tg/mL leupeptin, ImM **EDTA** , 1 gg/mL pepstatin, and 0.2 MM PMSF.

Protein concentrations were determined using the...

15/3,KWIC/46 (Item 15 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00445243
COMPOSITIONS THAT BIND TO PANCREATIC CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS QUI SE FIXENT SUR LES CELLULES CANCEREUSES PANCREATIQUES ET LEUR MODE D'UTILISATION
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
WEINBERG David,
WALDMAN Scott A,
BARBER Michael T,
BISWAS Sanjoy,
Inventor(s):
WEINBERG David,
WALDMAN Scott A,
BARBER Michael T,
BISWAS Sanjoy,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9835707 A1 19980820
Application: WO 98US3168 19980218 (PCT/WO US9803168)
Priority Application: US 9738063 19970218
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM

BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 15668

Fulltext Availability:
Detailed Description

Detailed Description
... invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm **purothionine** (Mak and Jones; Can, J. Biochem.; Vol. 22; p.

83J; (1976); incorporated herein in its...pH 5.2 and concentrated in the dialysis bags to about 100 ml with dry **polyethyleneglycol** (PEG 8000), Precipitated contaminating globulin proteins are removed by centrifugation at 6000 Xg for 15...

15/3,KWIC/49 (Item 18 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00359094
PYRULARIA THIONIN CONTAINING IMMUNOTOXINS AND IMMUNOTOXIN-LIKE CONJUGATES
THIONINE PYRULARIA CONTENANT DES IMMUNOTOXINES AINSI QUE DES CONJUGUES SIMILAIRES AUX IMMUNOTOXINES
Patent Applicant/Assignee:
THERA PRO,
Inventor(s):
VERNON Leo P,
RAEL Eppie D,
GASANOV Sardar E,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9641608 A2 19961227
Application: WO 96US8811 19960605 (PCT/WO US9608811)
Priority Application: US 95479799 19950607
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP
KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD
SE SG SI SK TJ TM TR TT UA UG UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD
RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG
CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 12214

Fulltext Availability:
Detailed Description

Detailed Description
... cytoplasmic target and kill the cell

Another immunotoxin which has been developed contains the toxin **purothionin** , isolated from barley flour, conjugated to the monoclonal antibody 225.28S. Unlike the other toxins used to date, **purothionin** does not need to be internalized to be cytotoxic. Instead, **purothionin** binds to the cell membrane where it disrupts the phospholipid bilayer causing cell death. Thus, **purothionin** is never exposed to the proteases found in the cytosol and lysosomes of the host cell

Unfortunately, conjugated **purothionin** is approximately 10,000 times less toxic than the ribosome inactivating toxins (1). As a result, the **purothionin** immunotoxin is only ...the mixture

of anti-CD5 and PT in phosphate
buffer (pH 6.9, 1 mM **EDTA**) by cation exchange HPLC. SCX 83
C13-ETI Hydropore column was equilibrated with 0.02 M Na2HPO4,
pH 6.9, 1 mM **EDTA** . A salt gradient was established with 1 M
NaCl and monitored as indicated by broken...the toxin's binding site.
This is especially true given the
contradictory data for the **purothionin** immunotoxin

5.5.4. Immunotoxin Specificity
The specificity of PT immunotoxin for CD5+ cells was...M of
2-Iminothiolane-HCl in 0.02 M Na2HPO4 (pH 7.0), 1 mM
EDTA . Anti-CD5 at 104M was incubated at 37(deg) ...10-4 M of SPDP in
0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . The deriva
tized proteins were separated from the reactants by dialysis
against 0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . Derivatized PT and
anti-CD5 were incubated together overnight at room temperature
in 0.02 M Na2HPO4 (pH 8.0), 1 mM **EDTA** . Unreacted PT was removed
by dialysis (14,000 MW cutoff) against water. The reaction
mixture...Company, Inc., Woburn, MA)
equilibrated with 0.02 M Na2HPO4 (pH 6.9), 1 mM **EDTA** . A salt
gradient was established with buffer containing 1 M NaCl (Fig
ure 4). Fractions...6). Samples for SDS-PAGE were
dissolved in buffer (20 mM Tris-HCl, 2 mM **EDTA** , 5% SDS, 0.01%
Bromophenol Blue) and applied to an 8-25% acrylamide gradient
gel...mM Tris-HCl buffer (pH 7.5) containing 0.1
M NaCl and 1 mM **EDTA** . 5-doxylstearic acid was used as a spin
probe. Oriented multibilayers were prepared by squeezing...of buffer (10
mM
Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM **EDTA**), mixing with a Vortex
mixer for 15 min. Multilamellar dispersions were incubated in
a helium...

15/3,KWIC/50 (Item 19 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00330288
NEUTRALIZATION OF FOOF ALLERGENS BY THIOREDOXIN
NEUTRALISATION D'ALLERGENES ALIMENTAIRES PAR LA THIOREDOXINE
Patent Applicant/Assignee:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
Inventor(s):
BUCHANAN Bob B,
KOBREHEL Karoly,
YEE Boihon C,
LOZANO Rosa,
FRICK Oscar L,
ERMEL Richard W,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9612799 A1 19960502
Application: WO 95US13206 19951018 (PCT/WO US9513206)
Priority Application: US 94326976 19941021
Designated States:
(Protection type is "patent" unless otherwise stated - for applications
prior to 2004)
AL AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG
KP KR KZ LK LR LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI
SK TJ TM TT UA UG UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT
LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 32222

Fulltext Availability:
Detailed Description

Detailed Description

GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX
NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI
FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 23391

Fulltext Availability:
Detailed Description

Detailed Description
... cyclophosphamide), cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics 5 include: **purothionin** (barley flour oligopeptide), macromomycin. 1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...any material capable of
binding proteins. Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the
protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** -tetraacetic acid (**EDTA**). One skilled in the art would readily recognize other fluorescence-emitting metals as well as...methotrexate, doxcrubicin, dauncrubicin, cytosinarabinoside,
etoposide, 5-4 fluorouracil, melphalan, chlorambucil, Cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...

15/3,KWIC/47 (Item 16 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00416399
PEPTIDE WITH INHIBITORY ACTIVITY TOWARDS PLANT PATHOGENIC FUNGI
PEPTIDE POSSEDANT UNE ACTION INHIBITRICE A L'ENCONTRE DE CHAMPIGNONS PATHOGENES DE PLANTES
Patent Applicant/Assignee:
NOVARTIS AG,
VERNOOIJ Barnardus Theodorus Maria,
CLARE Debra Arwood,
CHANDLER Danielle Brost,
KRAMER Catherine Mae,
Inventor(s):
VERNOOIJ Barnardus Theodorus Maria,
CLARE Debra Arwood,
CHANDLER Danielle Brost,
KRAMER Catherine Mae,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9806860 A1 19980219

Application: WO 97EP4438 19970813 (PCT/WO EP9704438)
Priority Application: US 9623940 19960814
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 20033

Fulltext Availability:
Detailed Description

Detailed Description
... were pipetted onto these disks. Test solutions were Jactoferrin (10 microgram) lactoferricin B (10 microgram), **purothionin** (the positive control, at 0, 5t 10 and 20 microgram; purchased from Calbiochern) and buffer...

...5 days, fungal growth was clearly visible on the plates, except around the filterdiscs containing **purothionin** and lactoferricin B. The sizes of the inhibition zones are shown in 1. In the...
...microgram lactoferricin B showed a zone of inhibition of 4.95 mm, whereas 20 microgram **purothionin** showed an inhibition zone of 4.5 mm. In the C. graminicola experiment, lactoferricin B produced a zone of inhibition of 1.7 mm, whereas **purothionin** produced an inhibition zone of 2.1 mm. Lactoterrin did not cause inhibition of fungal...

...25
Table 1: Inhibition of spore germination by lactoferricin B
zone of inhibition
Funaus Buffer **Purothionin** Lactoterricin B
20gg logg
0. maydis 0 mm 4.5 mm 4.95 mm
C...was added (0. 1 M LiClj 1 00 mM Tris pH 80 1 0 mM **EDTA** , 1% SIDS), followed by e qual volumes of water-saturated phenol and chloroform.

The RNA...

15/3,KWIC/48 (Item 17 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00394280 **Image available**
ALTERATION OF AMINO ACID COMPOSITIONS IN SEEDS
MODIFICATION DE COMPOSITIONS D'ACIDES AMINES DANS DES GRAINES
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
JUNG Rudolf,
HASTINGS Craig,
COUGHLAN Sean,
HU David,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9735023 A2 19970925
Application: WO 97US4409 19970319 (PCT/WO US9704409)
Priority Application: US 96618911 19960320
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN GH KE LS MW SD SZ UG AM AZ

... are soluble cereal seed proteins,
rich in cystine. In the Johnson, et al,
investigation, wheat **purothionin** was experimentally
reduced by NADPH via NADP-thioredoxin reductase (NTR)
and thioredoxin h according to Eqs, 2 and 3,
NTR,
(2) NADPH + Thioredoxin @ - NADP +
L&OX
Thioredoxin
(3) **Purothionin** ., + Thioredoxin hrw -+ **Purothioninrd**
+ Thioredoxin
Cereal seeds such as wheat, rye, barley, corn,
millet, sorghum and rice contain four...measures are taken to minimize
shock, renal
failure and respitory failure, Other than
administering calcium- **EDTA** in the vicinity of the
bite and excising the wound area, there are no known...PAGE
(Coomassie Blue stain), but in certain preparations,
the band was not sharp,
Other vroteins
Purothionin a from bread wheat and **purothionins** a-1
and fl from durum wheat were kind gifts from Drs, D.D,
Kasarda and B.L, Jones, respectively. The
purothionin a sample contained two members of the
purothionin family when examined with
SDS-polyacrylamide gel electrophoresis. The
purothionin a-1 and 0 samples were both homogeneous
in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried out in 100 mM potassium phosphate buffer,
pH 7,1, containing 10 mM **EDTA** and 16% glycerol in a
final volume of 0.1 ml. As indicated, 0,7...extraplastidic proteins,
this test has proved useful in several studies, A
case in point is **purothionin** which, when reduced by
thioredoxin h activates chloroplast FBPase (Wada, K,
et al. (1981), FEBS...

...and DSG-2) were found to be effective in
enzyme activation; however, they differed from
purothionin in showing a specificity for NADP-MDH
rather than FBPase (Table I). The a-amylase...2 0
ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
Thionins
** **Purothionin** -al 6 4 1 39
** **Purothionin** @P 6 ...same as for
the DSG/DTNB assay except that the DSG proteins were
omitted and **purothionin** a, 20 Ag or CM-1, 20 Ag was
used), The results thus confirmed the...a
thioredoxin requirement for reduction (data not
shown).

In confirmation of earlier results, thioredoxin
reduced **purothionin** consistently activated FBPase and
the type tested earlier, **purothionin** -a, failed to
activate NADP-MDH (Table I) (Wada, K., et al, (1981),
FEBS Lett. 124:237-240), However, in contrast to
purothionin -a from bread wheat, two **purothionins**
previously not examined (**purothionins** a-1 and a from
durum wheat) detectably activated NADP-MDH (Table I),
The two durum wheat **purothionins** also differed in
their ability to activate FBPase, The activity
differences between these **purothionins** were
unexpected in view of the strong similarity in their
amino acid sequences (Jones, B...

...undergo reduction by thioredoxin, A requirement for thioredoxin was observed for the reduction of -39@
purothionin (here the a@type) by the SDS-PAGE fluorescence procedure.

EXAMPLE 6
Ouantitation of Reduction...Procedure
The following concentrations of proteins were used (nmoles): thioredoxin, 0.08; NTRJV 0.01; **purothionin** fl, 1*7; DSG-1j 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...

...difference, other conditions were as in Examples 1
Reduction After
Protein 20 min 120 min
Purothionin -0 15 32
DSG-1 2 2 38
Corn kernel trypsin
inhibitor 3 15
Bowman...

...CM-1
a@amylase inhibitors (147 and 210%, respectively); corn kernel trypsin inhibitor (424%); and **purothionin** (82, 133, and 120% for the a, al and # forms, respectively). Glutaredoxin was ineffective in...

...and ovomucoid inhibitor), Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-amylase 5 inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 300C for 2 hours. The concentrations of thioredoxin, NTR, and NADPH were 0.024 mg/mlt OeO2 mg/ml, and 0.25 mM, respectively. With DTT as reductant, **EDTA** and components of the NADP/ thioredoxin system were omitted. Following reduction, aliquots ...HR (30 mM Tris-HCI, pH 7.5, containing 200 mM NaCl and 1 mM **EDTA**) chromatography, Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...30 mM Tris-HC1, pH 7.5, containing 200 mM Na Cl and 1 mM **EDTA** , Fractions (3.6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

15/3,KWIC/51 (Item 20 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00326460
COMPOSITIONS AND METHODS FOR THE ABROGATION OF CELLULAR PROLIFERATION UTILIZING THE HUMAN IMMUNODEFICIENCY VIRUS Vpr PROTEIN
COMPOSITIONS ET PROCEDES PERMETTANT D'INTERROMPRE UNE PROLIFERATION CELLULAIRE A L'AIDE DE LA PROTEINE Vpr DU VIH
Patent Applicant/Assignee:
THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
APOLLON INC,
WEINER David B,
LEVY David N,

REFAELI Yosef,
WILLIAMS William V,
AYYAROO Velpandi,
Inventor(s):
WEINER David B,
LEVY David N,
REFAELI Yosef,
WILLIAMS William V,
AYYAROO Velpandi,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9608970 A1 19960328
Application: WO 95US12344 19950921 (PCT/WO US9512344)
Priority Application: US 94309644 19940921
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 19863

Fulltext Availability:
Detailed Description

Detailed Description
... capable of binding antigen or antibodies. well-known supports or carriers, include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modif ied cellulose, polyacrylamide, agarose, and magnetite.

The nature of...metals can be attached to the TNF specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediaminetetraacetic** acid (**EDTA**).

The antibody also can be detectably labeled by coupling to a chemiluminescent compound. The presence...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...plus protease inhibitors: aprotinin, leupeptin, pepstatin A, each at 2 gg/ml; PMSF, 1mM, and **EDTA** , 1mM). Cell suspension was incubated on ice for 10 minutes with frequent vortexing, and centrifuged...then centrifuged at 10,000 G. These supernatants were supplemented - 42 with protease inhibitors (PMSF, **EDTA** , EGTA, aprotinin, pepstatin A, and Leupeptin), dialyzed against PBS, then filtered sterilized and kept on...12% glycerol, 12mM HEPES (pH 7.9), 4mM Tris (pH 7.9) , 6OmM KCI, 1mM **EDTA** , and 1mM DTT. The oligonucleotides sequences used were obtained from D. Ghosh.

The ...non-denaturing polyacrylamide gel (270 Al 1M Tris, pH 7.9; 80 til O.SM **EDTA** , pH 7.9; 13.2 Al 1M sodium acetate, pH 7.9; 5.33 ml...

...7. 9; 13. 2 ml 1M sodium Acetate, pH 7. 9; 8 ml 0. 5M **EDTA** , pH 8. 0; up to a final volume of 4 liters with ddH20). Gels were...

15/3,KWIC/52 (Item 21 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00300078
ANTIMICROBIAL PROTEINS
PROTEINES ANTIMICROBIENNES
Patent Applicant/Assignee:
ZENECA LIMITED,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
OSBORN Rupert William,
REES Sarah Bronwen,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9518229 A1 19950706
Application: WO 94GB2766 19941219 (PCT/WO GB9402766)
Priority Application: GB 9326424 19931224
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR BY CA CN CZ FI GE HU JP KG KP KR KZ LK LT LV MD MG MN NO NZ
PL RO RU SI SK TJ TT UA US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR
IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 7917

Fulltext Availability:
Detailed Description

Detailed Description
... 533-539). Such
proteins, including Sia2 from sorghum and
SURTMM SHEET (RULE 26)
g-l- **purothionin** (g-1P) from wheat, are known to
inhibit insect a-amylase and may be toxic...containing 10 mM NaH 2PO 41
15 mm
Na2HPO 41 100 mM KC1, 2 mM **EDTA** , 2 mM thiourea, and
1 mM PMSF. The homogenate was squeezed through
cheesecloth and clarified...buffer
contained 200 mM Tris-HC1 (pH 8.3), it (w/v) SDS, 1
mM **EDTA** , 0.005t bromophenol blue and, unless
otherwise stated, it (w/v) dithioerythritol (DTE).
Two hundred...

15/3,KWIC/53 (Item 22 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00293545
COMPOSITIONS THAT SPECIFICALLY BIND TO COLORECTAL CANCER CELLS AND METHODS OF USING THE SAME
COMPOSITIONS SE FIXANT SPECIFIQUEMENT A DES CELLULES CANCEREUSES COLO-RECTALES ET PROCEDES D'UTILISATION
Patent Applicant/Assignee:
THOMAS JEFFERSON UNIVERSITY,
WALDMAN Scott A,
Inventor(s):
WALDMAN Scott A,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9511694 A1 19950504
Application: WO 94US12232 19941026 (PCT/WO US9412232)
Priority Application: US 93141892 19931026; US 94305056 19940913
Designated States:

(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW NL NO NZ PL PT RO RU SD SE SI SK TJ TT UA US US UZ VN KE MW SD SZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 42481

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... g.

cyclophosphamide) , cis-platinum, vindesine (and other vinca alkaloids), mitomycin and bleomycin. other chemotherapeutics include: **purothionin** (barley flour oligopeptide) , macromomycin.

1,4-benzoquinone derivatives and trenimon.

Toxins are useful as active...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 flucrouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 114 benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens... methotrexate, doxcrubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium...methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine, mitomycin, bleomycin, **purothionin** , macromomycin, 114 benzoquinone derivatives, trenimcn, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens...any material capable of binding proteins.

Well-known solid phase supports include glass, polystyrene, polypropylene, **polyethylene** , dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the...metals can be attached to the protein-specific antibody using such metal chelating groups as **diethylenetriaminepentaacetic** acid (DTPA) or **ethylenediamine** tetraacetic acid (**EDTA**) . One skilled in the art would readily recognize other fluorescence-emitting metals as well as...Compound 2-D13 comprises bleomycin conjugated to SEQ ID NO:2.

Compound 2-D14 comprises **purothionin** con3ugated to SEQ ID NO:2.

Compound 2-D15 comprises macromomycin conjugated to SEQ ID...4 hours at room temperature in 0.4 Tris-HCl, pH 8.0 and 1mM **EDTA** . Reduced toxins are desalted on a Sephadex G-25 column equilibrated in TES buffer and...receptor binding activity of the ST peptide.

"'In is rapidly and potently chelated by either **EDTA** (**ethylenediaminetetraacetic** acid) or DTPA (**diethylenetriaminepetaacetic** acid). DTPA is preferred over **EDTA** because the latter may be more unstable in vivo. The "'In-DTPA is converted to...

Claim
... methotrexate,
doxorubicin, daunorubicin, cytosinarabinoside, etoposide, 5-4
fluorouracil, melphalan, chlorambucil, cis-platinum, vindesine,
mitomycin, bleomycin, **purothionin** , macromomycin, 114
benzoquinone derivatives, trenimon, ricin, ricin A chain,
Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens...

15/3,KWIC/54 (Item 23 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00286605
ANTIMICROBIAL PROTEINS
PROTEINES ANTIMICROBIENNES
Patent Applicant/Assignee:
ZENECA LIMITED,
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
REES Sarah Bronwen,
Inventor(s):
BROEKAERT Willem Frans,
CAMMUE Bruno Philippe Angelo,
REES Sarah Bronwen,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9504754 A1 19950216
Application: WO 94GB1636 19940729 (PCT/WO GB9401636)
Priority Application: GB 9316158 19930804; GB 9317816 19930827
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR BY CA CN CZ FI GE HU JP KE KG KP KR KZ LK LT LV MD MG MN MW
NO NZ PL RO RU SD SI SK TJ TT UA US UZ VN AT BE CH DE DK ES FR GB GR IE
IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Fulltext Word Count: 11516

Fulltext Availability:
Detailed Description

Detailed Description
... extraction buffer
containing 10 mM NaH2PO4, 15 mM Na 2HPO4, 100 mm
KCl, 2 mM **EDTA** and 2 mM thiourea. After
extraction, the slurry was mixed in a WARING
blender and...sample
buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v)
SDS, mM **EDTA** , 0.005% bromophenol blue and, unless
otherwise ...from Amaranthus
caudatus, seeds (Broekaert et al, 1992,
Biochemistry, 31: 4308-4314) and of ss- **purothionin**
from wheat endosperm (another type of plant seed
protein with antimicrobial activity; Redman DG and...

...sensitive to
the presence of all tested cations, the activities
of Ace-AMP1 and ss- **purothionin** seem to be rather
cation-stimulated although not by Cat+. The
antagonistic effect of Ca2...200
(nd = not determined)
TABLE 3
Antifungal activity of Ace-AMP1, Ac-AMP1 and
ss- **purothionin** on Fusarium culmorum in synthetic
medium supplemented with different cations
IC50 (gg/ml)
SMF +50...

...2 6
Ac-AMP1 4 100 100 50 rt;200 rt;200 rt;200
ss- **purothionin** 4 2 3 2 2 2.5 35
EXAMPLE 8
Anti-bacterial and anti-yeast...

15/3,KWIC/55 (Item 24 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00286604
COMPOSITIONS OF FUSION PROTEINS CONTAINING METALLOTHIONEIN AND TARGETING-PROTEIN STRUCTURAL COMPONENTS
COMPOSITIONS DE PROTEINES DE FUSION CONTENANT DES COMPOSANTS STRUCTURAUX DE METALLOTHIONEINE ET DE PROTEINE DE CIBLAGE
Patent Applicant/Assignee:
UNIVERSITY OF NEW MEXICO,
Inventor(s):
ZAMORA Paul,
GRIFFITH Jeffery K,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9504753 A1 19950216
Application: WO 94US8689 19940804 (PCT/WO US9408689)
Priority Application: US 93104628 19930811
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Fulltext Word Count: 9406

Fulltext Availability:
Detailed Description

Detailed Description
... chelating groups that have been used or can be so used include polydentate carboxylic acids (**EDTA** , DPTA, and the ....polydentate polyamines (amino oxime), chelates containing both amide and sulfur groups [N,N'-bis(mercaptoacetamino) **ethylenediamine** ; diaminodithiol], and chelates containing carboxyl and sulfur groups (dimercaptosuccinic acid). The chelator may be labeled...the thionins, which are present in monocots as well as in dicots. Cereal seeds contain **purothionins** which are toxic to animals and have thioredoxin activity. This invention contemplates the use of...molecules. MT-TPA molecules are extracted from cellular pastes in an extraction buffer containing Tris, **EDTA** , lysozyme, and deoxycholate and the inclusion bodies collected by centrifugation. The inclusion bodies are then...with a variety of radiometal complexing agents such as glucoheptate, citric acid, tartrate, tartrate/phthalate, **ethylene** diamine tetraacetic acid, or other stabilizing and/or complexing agents known to those skilled in...

15/3,KWIC/56 (Item 25 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00267908 **Image available**
HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN
DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE
Patent Applicant/Assignee:
PIONEER HI-BRED INTERNATIONAL INC,
Inventor(s):
RAO A Gururaj,
BEACH Larry R,

Patent and Priority Information (Country, Number, Date):
  Patent: WO 9416078 A2-A3 19940721
  Application: WO 94US382 19940112 (PCT/WO US9400382)
  Priority Application: US 933885 19930113
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
  AT AU BB BG BR BY CA CH CN CZ DE DK ES FI GB HU JP KP KR KZ LK LU LV MG MN MW NL NO NZ PL PT RO RU SD SE SK UA UZ VN AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 5778
Fulltext Availability:
  Detailed Description

Detailed Description
... crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information.

Three-dimensional modeling of the protein...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

15/3,KWIC/57 (Item 26 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00234016
USE OF THIOL REDOX PROTEINS FOR REDUCING DISULFIDE BONDS
UTILISATION DE PROTEINES D'OXYDOREDUCTION A BASE DE THIOL POUR REDUIRE DES LIAISONS BISULFURES
Patent Applicant/Assignee:
  THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
  BUCHANAN Bob B,
  KOBREHEL Karoly,
  YEE Boihon C,
  WONG Joshua H,
  LOZANO Rosa,
  JIAO Jin-an,
  SHIN Sungho,
Inventor(s):
  BUCHANAN Bob B,
  KOBREHEL Karoly,
  YEE Boihon C,
  WONG Joshua H,
  LOZANO Rosa,
  JIAO Jin-an,
  SHIN Sungho,
Patent and Priority Information (Country, Number, Date):
  Patent: WO 9308274 A1 19930429
  Application: WO 92US8595 19921008 (PCT/WO US9208595)
  Priority Application: US 91109 19911012; US 922 19920825
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
  AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO PL RO RU SD SE US US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG
Publication Language: English
Fulltext Word Count: 33674

Fulltext Availability:

Detailed Description

Detailed Description
... are
soluble cereal seed proteins, rich in cystine, In the Johnson, et al, investigation, wheat **purothionin** was experimentally reduced by NADPH via NADP-thioredoxin reductase (NTR) and thioredoxin h according to Eqs. 2 and 3,
(2) NADPH + Thioredoxin h NTR I NADP + Thioredoxin h,,
@d
(3) **Purothioninox** + Thioredoxin hd -+ **Purothionin** ,, + Thioredoxin hox
Cereal seeds such as wheat, rye, barley, corn, millet, sorghum and rice contain...measures are taken to minimize shock, renal failure and respitory failure, Other than administering calcium- **EDTA** in the vicinity of the bite and excising the wound area, there are no known...the E, coli NADP/Thioredoxin System.

Figs 5 is a graph showing the effect of **purothionin** a and CM-1 a-Amylase Inhibitor from Bread Wheat on DTNB Reduction by the...PAGE (Coomassie Blue stain) but in certain preparations, the band was not sharp.

Other proteins
Purothionin a from bread wheat and **purothionins** a-1 and from durum wheat were kind gifts from Drs. D, D, Kasarda and B.L. Jones, respectively, The purothionim a sample contained two members of the **purothionin** family when examined with SDS-polyacrylamide gel electrephoresis.

The **purothionin** a-1 and fl samples were both homogeneous in SDS-polyacrylamide gel electrophoresis.

Routine Method...

...was carried out in
100 mM potassium phosphate buffer, pH 7.1, containing 10 mM **EDTA** and 16% glycerol in a final volume of 0, 1 ml, As indicated, 0,7...extraplastidic proteins, this test has proved useful in several studies. A case in point is **purothionin** which, when reduced by thioredoxin h activates chloroplast FBPase (Wada, K. et ale (1981), FEBS...

...DSG-2) were f ound
to be effective in enzyme activation; however, they differed from **purothionin** in showing a specificity for NADP-MDH rather than FBPase (Table I), The a-amylase...ovoinhibitor 49 14 1 0
Bovine lung (Aprotinin) 7 3 Trace 2
3 0 Thionins
** **Purothionin** -al 6 4 1 39
** **Purothionin** -P 6 4 Trace 5
tPurothionin-a 6 4 0 14
These values compare to...

...5, conditions
were as in Fig. 4 except that the DSG proteins were omitted and **purothionin** ajr 20 Ag or CM-I,, 20 Ag was used) . The results thus confirmed the...thioredoxin in Fig, 7)e
EXAMPLE 5
Thioredoxin-linked Reduction of
Other Trypsin Inhibitors and **Purothionins**

In view of the finding that cystine-rich trypsin inhibitors from seeds can undergo specific...a thioredoxin requirement for reduction (data not shown) .

In confirmation of earlier results, thioredoxin@reduced **purothionin** consistently activated FBPase and the type tested earlier, **purothionin** -a , failed to activate NADP MDH (Table I) (Wada,, K.,, et al, (1981) , FEBS Lett, 124:237-240) . However,, in contrast to **purothionin** -a from bread wheat, two **purothionins** previously not examined (**purothionins** a-1 and 16 from duruin wheat) detectably activated NADP-MDH (Table I) , The two' durum wheat **purothionins** also differed in their ability to activate FBPase. The activity differences between these **purothionins** were unexpected in view of the strong similarity in their amino acid sequences (Jones . B...

...to undergo reduction by thioredoxin. A requirement for thioredoxin was- observed for the reduction of **purothionin** (here the a-type) by the SDS PAGE fluorescence procedure (Fig, 7),
EXAMPLE 6
Ouantitation...following concentrations of proteins were used (nmoles) : thioredoxin., 0 a 08; NTRI 0 e 01; **purothionin** @fl, T,
le7; DSG-Ir 0.7; corn kernel trypsin inhibitor, 1.0; Bowman-Birk...

...difference, other conditions were as in Fig. 6.
Reduction After
Protein 20 min 120 min
Purothionin -P 15 32
DSG-1 22 38
Corn kernel trypsin inhibitor 3 15
Bowman-Birk...

...CM-1 a-amylase inhibitors (147 and 210%,, respectively) ; corn kernel trypsin inhibitor (424%-) ; and **purothionin** (82, 133, and 120% for the a,, al and # forms, respectively) , Glutaredoxin was inef f...and ovomucoid inhibitor) . Those proteins that were reduced by either thioredoxin or glutaredoxin include the **purothionins** , two a-amylase inhibitors (DSG-1, CM-1), a cystine-rich trypsin inhibitor from plants...0.1 ml of 20 mM sodium phosphate buffer, pH 7.9 containing 10 mM **EDTA** at 300C for 2 hours. The concentrations of thioredoxin, NTR,, and NADPH were 0,024 mg/mIj, 0,02 mg/ml, and 0.25 mM, respectively. With DTT as reductant,, **EDTA** and components of the NADP/thioredoxin system were omitted. Following reduction, aliquots of the inhibitor...HR (30 mM Tris-HCl, pH 7.5, containing 200
mM NaCl and 1 mM **EDTA**) chromatography. Pullulanase inhibitor protein was purified as described below.

CM32 Chromatography
The pullulanase inhibitor sample...

...30 mM Tris
HC11 pH 7,5, containing 200 mM Na Cl and 1 mM **EDTA** , Fractions (3*6 ml/fraction) showing pullulanase inhibitory activity were pooled, concentrated by dialysis against...

15/3,KWIC/58 (Item 27 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00230900

BIOCIDAL PROTEINS

PROTEINES BIOCIDES

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Patent and Priority Information (Country, Number, Date):
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Priority Application: GB 9118523 19910829; GB 923038 19920213; GB 9213526 19920625

Designated States:
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AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG

Publication Language: English
Fulltext Word Count: 13654

Fulltext Availability:
Detailed Description
Claims

Detailed Description

... 1990 Eur J Biochem, 194:533-539). Such proteins, including SI=2 from sorghum andy **purothionin** from wheat, are known to inhibit insect a-amylase and are toxic to insect larvae...Rs-AFPI, Dm-AMP1. the Cb-AMPs, Lc-AFP, Ct-AMPI, sorghum Sia2, wheat Y1 **purothionin** , and the predicted products of the pea genes p1230 and pI39, of the cowpea gene...buffer containing 10 mM NaH2PO VP 15,mM Na2HPO 4J' 100 mm KC11 2 mM **EDTA** , 2 mK thiourea, and 1 mN PMSF. The homogenate was squeezed through cheesecloth and clarified...NaH PO 15 mM Na HPO 100 mm 2 41 2 41 KClr 2 mM **EDTA** and 1 mM benzamidine. The resulting homogenate was squeezed through cheesecloth and clarified by centrifugation...buffer contained 200 mM Tris-HCl (pH 8,3), 1% (w/v) SDS, 1 mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dit loerythritol (DTE).

Proteins...6 M guanidinium-Cl containing 100 mm sodium phosphate buffer (pH 7) and 1 mM **EDTA** , The mixtures were allowed to react with 5,5t-dithionitrobenzoic acid and monitored for release...Sorghum bicolor (Bloch and Richardson, 1991, FEBS Lett, 279, 101-104), and also to y- **purothionins** from Triticum aestivum (Colilla et al, 1990, FEBS Lett,

270, 191-194) which inhibit 'in...

...the Cb-AMPs,
Lc-AFP, Ct-AMP1, the sorghum a-amylase inhibitor
SIa2, wheat yl **purothionin** , and the predicted
sequences of the mature protein products of the
Fusarium-induced pea genes...supplement, respectively, For the purpose
of comparison, these tests were performed in
parallel with 0- **purothionin** , an antifungal protein
from wheat seeds (isolated as described in Redman
and Fisher, 1969, J...

...more than 30-fold with both test fungi,
in comparison, the IC50 value of 0- **purothionin**
TABLE 6
VARIATIONS IN ANTIFUNGAL ACTIVITY IN THE PRESENCE OF X+ AND
Fungus Antifungal IC50...

...6 10
Rs-AFP2 3 2 2 2
Rs-nsLTP 20 35 >1000 108
0- **purothionin** 10 7 4 10
Mj-AMP2 4 5 40 50
T hamatum Rs-AFP1 7...

...7 30
Rs-AFP2 2 2 3 2
Rs-nsLTP 30 60 >1000 >1000
P- **purothionin** 4 3 1e5 4
Mj-AMP2 2 2 25 20
increased by about 7-fold...AFP2,,
nor Rs-nsLTP affected cell viability after 24 h of
incubation. In-contrast, 0- **purothionin**
administered at 50 pg/ml decreased the viability of
both cell types by more than...

Claim
... pI322.
A process of combating fungi or bacteria which
comprises exposure to SIm2, y-1- **purothionin** ,
or another v.-amylase inhibitor protein.
An extraction process for producing a protein
as claimed...

15/3,KWIC/59 (Item 28 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00230335
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
Patent Applicant/Assignee:
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Patent and Priority Information (Country, Number, Date):
Patent: WO 9304586 A1 19930318
Application: WO 92GB1574 19920828 (PCT/WO GB9201574)
Priority Application: GB 9118730 19910902
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO RU SD US AT BE CH DE DK ES FR GB GR IE IT LU MC NL SE BF BJ CF CG CI CM GA GN ML MR SN TD TG
Publication Language: English
Fulltext Word Count: 9050

Fulltext Availability:
Detailed Description

Detailed Description
... buffer containing-10 mM NaH2 PO 4" 15 mK Na2HP041 100 mM KClf 2 mM **EDTAr** 2 mM thiourea and I mM PMSF. The homogenate was squeezed through cheesecloth and clarified...proteins contained 200 mm Tris-HCl (pH 8-3), 1% (w/v) SDS, 1 mM **EDTA** , 0*005% bromophenol bluer and the sample buffer for analysis of reduced proteins contained a...and napin sequences.
EXAMPLE 3
Isolation of the trypsin inhibitors from barley seeds and cc- **purothionins** from wheat seeds.

It is known from the literature that barley (Hordeum vulgare L.) contains...N-terminal amino acid sequences of the trypsin inhibitors from barley and from WG11.

a- **Purothionin** was isolated from wheat (Triticum aestivum) seed flour essentially as described by Redman and Fisher...

...a linear gradient (40 min) from 0 to 40% acetonitrile in 0.1% TFA. The **purothionineluted** was two :incompletely resolved peaks at 34 and 35% acetonitrile, respectively, which represent the ml...

...Jones, 1977, Cereal Chem, 54, 511-523). Both isoforms were pooled, to yield the a- **purothionin** preparation.,
EXAMPLE 4
Anti-fungal activity assay.

Antifungal activity was measured by microspectrophotometry as previously...5
Antifungal activity of the radish and rapeseed 2S albumins, barley trypsin inhibitors and a- **purothionin** .

The antifungal potency of the radish 2S albumins, rapeseed 2S albumins, barley trypsin inhibitors and a- **purothionin** was assessed by the microspectrophotometric assay described in Example 4. Growth of fungi, collection and...

...1
ANTIFUNGAL ACTIVITY OF 2S ALBUMINS FROM RAPESEED AND RADISHe BARLEY I INHIBITORS AND a- **PUROTHIONIN**
Protein IC 50 (pg/ml)
Medium A Mediuin B
Fc Ab Ap Vd Fc Ab...

...65 >1000 >1000 >1000 >101
bti2 90 40 90 50 >1000 >1000 >1000 >101
a- **purothionin** 4 4 8 2 11 11 12
In medium A (low ionic strength), the 2S albumins from rapeseed and radish, the barley trypsin inhibitors btil and bti2 and a.- **purothionin** were active on all four fungi tested. The five isoform fractions of the radish 2S...inhibitors were completely inactive at concentrations up to 1 mg/ml. In contrast the a- **purothionin** remained active in medium B, although its specific activity was decreased by about two- to...

...30-fold on T hamatum,
EXAMPLE 7
Synergism between 2S albumins, barley trypsin inhibitor and =@- **purothionin** .

The synergistic antifungal effect of combinations of 2S albumins and a- **purothionin** , on the one hand, and barley trypsin inhibitors and m- **purothionin** , on the other hand, was measured as follows.

To serial dilutions of m- **purothionin** , a constant subinhibitory concentration of 2S albumins or barley trypsin inhibitors was added. The IC so value of the a- **purothionin** was calculated from dose-response curves for the series with and without addition of 2S 3
SYNERGISTIC ANTIFUNGAL EFFECT OF COMBINATIONS BETWEEN 2S ALBUMINS/a@ **PUROTHIONIN** AND TRYPSIN INHIBITORS/=- **PUROTHIONIN**
Fungus Test IC 50 of a- **purothionin** in #g/ml
Protein (Synergism Factor)
Medium A Medium B
Test protein Test protein
conc...

...The highest synergism factor (73) was obtained in medium B for the combination of a.- **purothionin** and Bn-2S3 (at 250 jig/ml) on the fungus Alternaria brassicola. The synergism factors...

...2S albumins
Rs-2S3 and Bn-2S3.

EXAMPLE 8
Effect of 2S albumins and a- **purothionin** on bacterial growth.

Antibacterial activity was measured micro5pectrophotometrically as follows. A bacterial suspension was prepared...these bacteria.

Synergisms in antibacterial activity were assessed for combinations between Rs-2S3 and a.- **purothionin** by using the same approach as described in Example 7. A synergistic effect was observed...

...of 10, 50 and 250 jig/ml, respectively, to a serial dilution series of a- **purothionin** (Table 4). Thus, the thionin-potentiating activity of 2S albumin is not limited to fungi...
...TABLE 4
SYNERGISTIC ANTIBACTERIAL EFFECT ON

BACILLUS MEGATERIUM OF COMBINATIONS BETWEEN
RS-2S3 AND a- **PUROTHIONIN**
Rs-2S3 conc. IC so of a- **purothionin** in #g/ml
(/Jg/ml) (Synergism Factor)
Medium C Medium D
2 2*5
0...

...Oe6(4)
Oo2(15)
250 Oe15(17)
EXAMPLE 9
Effect of 2S albumins and w- **purothionin** on
cultured human cells.

Human cell toxicity assays were performed
either on umbilical vein endothelial...Rs-2S3 did not affect cell
viability after 24 hours of incubation. in
contrast, cc- **purothionin** administered at 50 jig/ml
and 20 jig/ml decreased the viability of both cell...
...a constant concentration of
250 ug/ml to a serial dilution series of a
(x- **purothionin** did not increase the toxic activity
of the-a- **purothionin** .

EXAMPLE 10
Antifungal activity of the small and large
subunit of 2S albumins.

The small...

...antifungaleffect on F
culmorum was assessed for combination between
oxidised SS or LS and a- **purothionin** by using the
same approach as described in Example 7. The
results of these experiments...

...SYNERGISTIC ANTIFUNGAL EFFECT ON FUSARIUN CULNORUN OF CONBINATIONS BI
OXIDISED SS OR LS AND w- **PUROTHIONIN**
Test protein IC 50 of a- **purothionin** , og/ml (Synergism Factor)
Nedium A Medium B
Test protein conc. Test protein conc.
(Pg...

...LS (though to
a much lesser extent) are able to potentiate the
activity of a.- **purothionin** , A synergism factor of
up to 33 could be obtained when oxidised SS was
added at 10 pg/ml to a lution of a.- **purothionin**
and assayed in medium A. In medium B a synergism
factor of 2 was measured...14576-14581) and also
exert antifungal activity and display the
synergistic effect with the a- **purothionin** (see
Examples 5 and 10), this substitution is not
believed to affect nor the antifungal...

15/3,KWIC/60 (Item 29 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00224457
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
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Patent and Priority Information (Country, Number, Date):
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Priority Application: GB 9112300 19910607
Designated States:
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AT AU BB BE BF BG BJ BR CA CF CG CH CI CM CS DE DK ES FI FR GA GB GN GR HU IT JP KP KR LK LU MC MG ML MN MR MW NL NO PL RO RU SD SE SN TD TG US
Publication Language: English
Fulltext Word Count: 7952

Fulltext Availability:
Detailed Description

Detailed Description
... buffer containing 10 MM NaH 2POV 15 mm Na2HPO 41 100 MM KC1, 2 MM **EDTA** , 2 MM thiourea, 1 MM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...buffer contained 200'mM TriS-HC1 (pH 8*3), 1% (w/v) SDSI I mM **EDTA** , 0,005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT), Proteins were...

...6 M guanidinium-Cl containing 100 mM sodium phosphate buffer (pH 7) and I mM **EDTA** , The mixtures were allowed to react with 5,51-dithionitrobenzoic acid and monitored for release...AMP1 nor Ac-AMP2 affected cell viability after 24 h of incubation. In contrast, A- **purothionin** administered at 50 pg/ml decreased the viability of both cell types by more than...

15/3,KWIC/61 (Item 30 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00218464
BIOCIDAL PROTEINS
PROTEINES BIOCIDES
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REES Sarah Bronwen,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9215691 A1 19920917
Application: WO 92GB423 19920310 (PCT/WO GB9200423)
Priority Application: GB 915052 19910311; GB 915684 19910319

Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AT AT AU BB BE BF BG BJ BR CA CF CG CH CH CI CM CS DE DE DK DK ES ES FI FR GA GB GB GN GR HU IT JP KP KR LK LU LU MC MG ML MN MR MW NL NL NO PL RO RU SD SE SE SN TD TG US
Publication Language: English
Fulltext Word Count: 6953

Fulltext Availability:
Detailed Description

Detailed Description
... extraction buffer containing 10 mM NaH2POV 15 mM Na 2HP041 100 mM KC1. 2 mM **EDTA** , 2 mM thiourea, I mM PMSF and 1 mg1l leupeptin. The homogenate was squeezed through...buffer contained 200 mM Tris-HCI (pH 8.3), 1% (w/v) SDS, I mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and 0- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...

...as previously described (Peumans, Wi et al; 1983; FEBS Lett, 177, 99-103). The 0- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP21 UDA and 0- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...20 jig/ml for Mj-AMP2. from 0.5 to 15 jig/ml for a- **purothionin** , and from 20 to over 1,000 /ig/ml for UDA depending on the test...

...on an average basis the obtained antifungal activity series is as follows: Mj-AMP2 = 0- **purothionin** > Mj-AMP1 > UDA, Some fungi, such as B cinerea, C lindemuthianum and V inaegualis, are clearly more sensitive to Mj-AMP2 than to 0- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and P- **purothionin** than for Mj-AMP1 or UDA.

ALsor Mj-AMP2 and 0- **purothionin** characteristically produced steeper dose-response curves than Mi-AMP1 or UDA. Figure 5 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and a- **purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (0 ---- 0...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovorao A- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

...hours, Results are shown in Table 6.

TABLE 6
Antibacterial activity of Mj-AMPs, 9- **purothionin** and UDA
Bacterium IC 50 (/Jg/ml)
Mj-AMP1 Mj-AMP2 13-pt UDA

MEDIUM...

15/3,KWIC/62 (Item 31 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00208988
PROTEINACEOUS ANTI-DENTAL PLAQUE AGENTS
AGENTS D'ELIMINATION DE LA PLAQUE DENTAIRE A BASE DE PROTEINES
Patent Applicant/Assignee:
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Inventor(s):
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Patent and Priority Information (Country, Number, Date):
Patent: WO 9206191 A1 19920416
Application: WO 91US7099 19910927 (PCT/WO US9107099)
Priority Application: US 90657 19900928; US 91989 19910301
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AT AT AU BB BE BF BG BJ BR CA CF CG CH CH CI CM CS DE DE DK DK ES ES FI FR GA GB GB GN GR HU IT JP KP KR LK LU LU MC MG ML MN MR MW NL NL NO PL RO SD SE SE SN SU TD TG US
Publication Language: English
Fulltext Word Count: 13547

Fulltext Availability:
Detailed Description

Detailed Description
... human inter-alpha trypsin inhibitor (58AAF 3 -SS-), crambin (46 AA; 3 -SS-)f alpha **purothionin** (45 AA; 4 -SS-), beta **purothionin** (same), human secretory leukocyte protease inhibitor (107 AA; 8 -SS 30), hen egg-white lysozyme...of 0,1 to 12% by weight.

The composition may comprise a humectant, such as **polyethylene** glycol, **ethylene** glycol, sorbitol, glycerol,, propylene glycol, 1,3-butylene glycol, xylitol, maltilol, lactitol, and the like...tablet may optionally be coated with a coating material such as waxes, shellac, carboxymethyl cellulose, **polyethylene** /maleic anhydride co-polymer or Kappacarrageenan to further increase the time it takes the tablet...

15/3,KWIC/63 (Item 32 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00159749
USE OF THIOREDOXIN, THIOREDOXIN-DERIVED, OR THIOREDOXIN-LIKE DITHIOL PEPTIDES IN HAIR CARE PREPARATION
UTILISATION DE PEPTIDES DE DITHIOL DE THIOREDOXINE, DERIVES OU ANALOGUES DE THIOREDOXINE, DANS DES PREPARATIONS DE SOIN DES CHEVEUX
Patent Applicant/Assignee:
REPLIGEN CORPORATION,
Inventor(s):
PIGIET Vincent P,
Patent and Priority Information (Country, Number, Date):
Patent: WO 8906122 A1 19890713
Application: WO 88US4694 19881229 (PCT/WO US8804694)
Priority Application: US 88353 19880104; US 88354 19880104

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AT BE CH DE FR GB IT JP LU NL SE
Publication Language: English
Fulltext Word Count: 6278

Fulltext Availability:
Detailed Description

Detailed Description
... USA 75:5827-5830). Other thioredmin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredcLdn-like activity (Wada, K and Buchanan, B.B. [1983] in 'Thioredoxins...5 ml aliquots in -200C in O,5M Tris, pH 7.4 with 1 mM **EDTA** .

Thioredcodn protein is assayed immunologically using quantitative rocket immuncelectrophoresis, as described in McEvoy et al...column equilibrated with OAM Tris, pH 7.5, containing 0.5M NaCl and 1 mM **EDTA** .

The column was washed with two column volumes of the equilibrating buffer containing 2M urea...

...x 25 cm column of Sephadex"m G 40 equilibrated with 0.05M Tris, 1mM **EDTA** , pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280...7% (w/w) cammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) **polyoxyethylene** (23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. AU dilutions...30,0
Sodium carbonate glycinate 5eO
Ammonium thioglycollate or
thiolactate (50% aqueous
soln) 3,0
EDTA (disodium salt) 0,3
Sodium p-hydroxybenzoate
methyl ester 0,05
Monoethanolamine 10
Imidazoline. Os2...

15/3,KWIC/64 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0006236844 BIOSIS NO.: 198886076765
TYROSINE HYDROGEN-BONDING AND ENVIRONMENTAL EFFECTS IN PROTEINS PROBED BY UV RESONANCE RAMAN SPECTROSCOPY
AUTHOR: HILDEBRANDT P G (Reprint); COPELAND R A; SPIRO T G; OTLEWSKI J; LASKOWSKI M JR; PRENDERGAST F G
AUTHOR ADDRESS: DEP CHEM, PRINCETON UNIV, PRINCETON, NEW JERSEY 08544, USA **USA
JOURNAL: Biochemistry 27 (15): p5426-5433 1988
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

...ABSTRACT: chicken [OMCHI3(-)] and from chachalaca [OMCHA(-)], as well as .alpha.1-, .alpha.2-, and .beta.- **purothionin** . At this excitation wavelength interference from phenylalanine is minimized, and it is possible to determine...

...kcal/mol were found for OMCHA3(-) and for .alpha.1- (or .alpha.2-) and .beta.- **purothionin** , respectively. The intensity of the 1176-cm-1 .nu.9a band of Tyr excited at...
...correlate strongly with the estimated H-bond enthalpies, but large deviations are seen for the **purothionins** , reflecting a special

environment for the Tyr residue of these proteins, which is believed to ...

...phenylalanine in aqueous solution is about half the value observed in most proteins. Addition of **ethylene** glycol to aqueous phenylalanine increases the intensity, which attains a value similar to those seen...
DESCRIPTORS: CHICKEN CHACHALACA **PUROTHIONIN**

15/3,KWIC/65 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00642457
HIGH LYSINE DERIVATIVES OF ALPHA-HORDOTHIONIN
DERIVATE VON ALPHA-HORDOTHIONIN MIT HOHEREM BEHALT AN LYSIN
DERIVES D'ALPHA-HORDOTHIONINE A HAUTE TENEUR EN LYSINE
PATENT ASSIGNEE:
PIONEER HI-BRED INTERNATIONAL, INC., (1142078), 800 Capital Square, 400 Locust Street, Des Moines, Iowa 50309, (US), (Proprietor designated states: all)
INVENTOR:
RAO, A. Gururaj, 4628 70th Place, Urbandale, IA 50322, (US)
BEACH, Larry R., 3939 Maquoketa Drive, Des Moines, IA 50311, (US)
LEGAL REPRESENTATIVE:
Irvine, Jonquil Claire et al (74182), J.A. KEMP & CO. 14 South Square Gray's Inn, London WC1R 5JJ, (GB)
PATENT (CC, No, Kind, Date): EP 745126 A1 961204 (Basic)
EP 745126 B1 010912
WO 9416078 940721
APPLICATION (CC, No, Date): EP 94908585 940112; WO 94US382 940112
PRIORITY (CC, No, Date): US 3885 930113
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/29; C07K-014/00; C12N-005/10; A01H-005/00; A01N-065/00; C12N-001/21
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200137	515
CLAIMS B	(German)	200137	492
CLAIMS B	(French)	200137	578
SPEC B	(English)	200137	4391
Total word count - document A			0
Total word count - document B			5976
Total word count - documents A + B			5976

...SPECIFICATION crystal structures have not previously been available for hordothionin or even related compounds such as **purothionin** and viscotoxin. We undertook to develop such structural information.
Three-dimensional modeling of the protein...oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...

15/3,KWIC/66 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00579685
BIOCIDAL PROTEINS
BIOZIDE PROTEINE
PROTEINES BIOCIDES
PATENT ASSIGNEE:

Syngenta Limited, (1579446), Fernhurst, Haslemere, Surrey GU27 3JE, (GB),
(Proprietor designated states: all)
INVENTOR:
DE BOLLE, Miguel, Kleine Bareelstraat 15 Bus 201, B-2800 Mechelen, (BE)
BROEKAERT, Willem, Frans, Levoldlaan 18, B-1700 Dilbeek, (BE)
CAMMUE, Bruno, Philippe, Angelo, J.B. Woutersstraat 109A, B-1652 Alsemberg, (BE)
VANDERLEYDEN, Jozef, Kastanjelaan 12, B-3001 Heverlee, (BE)
REES, Sarah, Bronwen 32 Micheldever Way, Forest Park Bracknell, Berkshire RG12 3XX, (GB)
LEGAL REPRESENTATIVE:
Roberts, Timothy Wace et al (35342), Intellectual Property Department ZENECA Seeds Jealotts Hill Research Station P.O. Box 3539, Bracknell Berkshire RG12 6YD, (GB)
PATENT (CC, No, Kind, Date): EP 576483 A1 940105 (Basic)
EP 576483 B1 010816
WO 9215691 920917
APPLICATION (CC, No, Date): EP 92906477 920310; WO 92GB423 920310
PRIORITY (CC, No, Date): GB 9105052 910311; GB 9105684 910319
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; MC; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/82; C07K-014/415; C12N-015/29; A01H-005/00; A01N-065/00; A01N-063/02; A61K-038/00
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200133	408
CLAIMS B	(German)	200133	344
CLAIMS B	(French)	200133	431
SPEC B	(English)	200133	5803
Total word count - document A			0
Total word count - document B			6986
Total word count - documents A + B			6986

...SPECIFICATION buffer containing 10 mM NaH2))PO4)), 15 mM Na2))HPO4)), 100 mM KCl, 2 mM **EDTA** , 2 mM thiourea, 1 mM PMSF and 1 mg/l leupeptin. The homogenate was squeezed...buffer contained 200 mM Tris-HCl (pH 8.3), 1% (w/v) SDS, 1 mM **EDTA** , 0.005% bromophenol blue and, unless otherwise stated, 1% (w/v) dithiothreitol (DTT). Silver staining...dioica agglutinin or UDA (Broekaert, WF et al; 1989; Science, 245, 1100-1102) and (beta)- **purothionin** (Hernandez-Lucas, C et al; 1974; Appl Microbiol, 28, 165-168). Fungi were grown on...as previously described (Peumans, WJ et al; 1983; FEBS Lett, 177, 99-103). The (beta)- **purothionin** was purified from wheat endosperm by the method of Redman, DG and Fisher, N (1969...

...Table 2 summarises the results. Serial dilutions of Mj-AMP1, Mj-AMP2, UDA and (beta)- **purothionin** were applied to fungi and the percent growth inhibition measured by microspectrophotometry (as described in...

...g/ml for Mj-AMP2, from 0.5 to 15 (mu)g/ml for (beta)- **purothionin** , and from 20 to over 1,000 (mu)g/ml for UDA depending on the...

...On an average basis the obtained antifungal activity series is as follows: Mj-AMP2 = (beta)- **purothionin** > Mj-AMP1 > UDA. Some fungi, such as B cinerea, C lindemuthianum and V inaequalis, are clearly more sensitive to Mj-AMP2 than to (beta)- **purothionin** . Conversely, the latter protein is most effective in deterring growth of other fungi such as...

...time-dependent drop in antifungal activity, however, was less pronounced for Mj-AMP2 and (beta)- **purothionin** than for Mj-AMP1 or UDA. ALso, Mj-AMP2 and (beta)- **purothionin** characteristically produced steeper dose-response curves than Mj-AMP1 or UDA. Figure 5 shows the...

...and B), Mj-AMP2 (panels C and D), UDA (panels E and F), and (beta)-**purothionin** (panels G and H). The percent growth inhibition was recorded after 48 h (*----*), after 60...positive and gram-negative bacteria: Bacillus megaterium, Sarcina lutea, Escherichia coli and Erwinia carotovora. (beta)- **purothionin** and UDA were also tested for comparison (see Example 7). Tests were performed in soft...

15/3,KWIC/67 (Item 3 from file: 348)

00537586

Natural and synthetic proteins with inhibitory activity towards pathogenic microorganisms.

Naturliche und synthetische Proteine mit inhibitorischer Aktivitat gegen pathogene Mikroorganismen.

Proteines naturelles et synthetiques avec activite inhibitrice contre des microorganismes pathogenes.

PATENT ASSIGNEE:
PIONEER HI-BRED INTERNATIONAL, INC., (1142071), 700 Capital Square 400 Locust Street, Des Moines Iowa 50309, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Duvick, Jonathan, 1707 38th Street, Des Moines, Polk County, Iowa 50310, (US)
Rood, Tracy, 4333 Allison Avenue, Des Moines, Polk County, Iowa 50310, (US)
LEGAL REPRESENTATIVE:
Goldin, Douglas Michael et al (31062), J.A. KEMP & CO. 14, South Square Gray's Inn, London WC1R 5LX, (GB)

PATENT (CC, No, Kind, Date): EP 502718 A1 920909 (Basic)
APPLICATION (CC, No, Date): EP 92301868 920304;
PRIORITY (CC, No, Date): US 664270 910304
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: A01N-037/46; A01N-037/44; A01N-063/00; A01N-065/00; C12N-015/00; C12N-015/52;
ABSTRACT WORD COUNT: 54

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	959
SPEC A	(English)	EPABF1	5135
Total word count - document A			6094
Total word count - document B			0
Total word count - documents A + B			6094

...SPECIFICATION oil, corn oil and soybean oil; polyols such as propylene glycol, glycerin, sorbitol, mannitol and **polyethylene** glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium...hormone 1-24, Citrate synthase, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Crotamine, Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.
Preferred proteins for...

...hormone 1-24, Citrate synthase, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.
Preferred proteins for killing...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Colletototrichum graminicola are: Magainin-A...

...L-Lysine HCl, poly-D-Lysine, poly-D- Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Verticillium albo-atrum are: Magainin...

...L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Phytophthora megaspermae f.sp. glycinea are: Magainin-2, poly-L-Lysine HBr, Defensin NP1, b- **purothionin** and Melittin.
Preferred proteins for killing or inhibiting the pathogen Macrophomina phaseolina are: poly-L-histidine, poly-D-Lysine, poly-D-Lysine HBr, Defensin NP1, a-hordothionin and b- **purothionin** .
Preferred proteins for killing or inhibiting the pathogen Diaporthe phaseolorum caulivora are: Defensin NP1, a-hordothionin and b- **purothionin** .
Preferred proteins for killing or inhibiting the pathogen Sclerotinia sclerotiorum are: Magainin-A, Magainin-G...

...poly-D-Lysine HBr, Mastoparan, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , stinging nettle lectin, Crotamine, Melittin, Eosinophil major basic protein and Eosinophil cationic protein.
Preferred proteins...

...poly-D-Lysine HBr, Mastoparan, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Stinging nettle lectin, Crotamine, Melittin, Eosinophil major basic protein and Eosinophil cationic protein.
Preferred proteins...
...D-Lysine HBr, Mastoparan, Kassinin, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Stinging nettle lectin, Crotamine, Melittin and Eosinophil cationic protein.
Preferred proteins for killing or inhibiting...

...hormone 1-24, Citrate synthase, Defensin NP1, Cathepsin G, Lysozyme, a-hordothionin, b-hordothionin, b- **purothionin** , Crotamine, Melittin, Eosinophil major basic protein, Eosinophil cationic protein, and Eosinophil peroxidase.
Example 2 - Protein...proteins and enzymes Citrate Synthase, Defensin 1, Cathepsin G, Lysozyme, a-Hordothionin, b-Hordothionin, b- **Purothionin** , Stinging Nettle Lectin, Crotamine, Melittin, Eosinophil Major Basic Protein, Eosinophil cationic Protein and Eosinophil Peroxidase...

...CLAIMS selected from: Defensin NP1, Magainin-2, Magainin-A, Magainin-G, a-Hordothionin, b-Hordothionin, b- **Purothionin** , poly-L-Lysine HBr, poly-L-Lysine HCl, poly-D-Lysine, poly-D-Lysine HBr...

15/3,KWIC/68 (Item 4 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00245328
Therapeutic and related uses of dithiol peptides.
Therapeutische und verwandte Verwendungen von Dithiol-Peptiden.
Utilisations therapeutiques et apparentees des dithiol peptides.
PATENT ASSIGNEE:
REPLIGEN CORPORATION, (545550), 101 Binney Street, Cambridge Massachusetts 02142, (US), (applicant designated states: BE;CH;DE;ES;FR;GB;GR;IT;LI;NL;SE)
INVENTOR:
Pigiet, Vincent P., 666 Main Street, Apt. L-1, Winchester Massachusetts 01898, (US)
Millis, Cynthia D., 3720 West 8th Avenue, Apt. 5, Vancouver British Columbia, V6R 1Z3, (CA)

LEGAL REPRESENTATIVE:
  Perry, Robert Edward et al , GILL JENNINGS & EVERY 53-64 Chancery Lane, London WC2A 1HN, (GB)
PATENT (CC, No, Kind, Date): EP 237189 A2 870916 (Basic)
APPLICATION (CC, No, Date): EP 87301150 870210;
PRIORITY (CC, No, Date): US 839857 860314; US 921287 861020; US 828112 860210
DESIGNATED STATES: BE; CH; DE; ES; FR; GB; GR; IT; LI; NL; SE
INTERNATIONAL PATENT CLASS: A61K-037/02;
ABSTRACT WORD COUNT: 18

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	285
SPEC A	(English)	EPABF1	3897
Total word count - document A			4182
Total word count - document B			0
Total word count - documents A + B			4182

...SPECIFICATION i.e., rates of reaction of uncatalyzed reactions are slow). Certain iron-chelators such as **ethylenediaminetetraacetic** acid (**EDTA**) promote the iron-catalyzed Haber-Weiss reaction presumably by chelating the Fe(sup 3)(sup...or one that is readily soluble can be utilized. For example, cocoa butter and various **polyethylene** glycols (Carbowaxes) can serve as the vehicle.
  For intranasal instillation, fluid unit dosage forms are...USA, 75, 5827-5830). Other thierodoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. (1983) in "Thioredoxins...show that thioredoxin chelates iron with an affinity comparable to or better than that of **EDTA** . Similar results were also observed with copper.
  Example 4
  Upon substituting the thioredoxin in Examples...

...essentially the same results.
  Example 6
  Thioredoxin was compared to several iron chelators (i.e., **EDTA** and desferrioxamine) in its ability to prevent radical formation. The iron caralyzed formation of the...

...M inhibited all catalytic activity of the iron in that no formaldehyde was detected, whereas **EDTA** at 250 (mu)M gave a rate of 7.1 (+-) 0.8 nmols formaldehyde/30...

...nmols of formaldehyde/30 min. Thus thioredoxin behaves as desferrioxamine in inhibiting radical formation whereas **EDTA** cannot.
  Example 7
  Thioredoxin inhibited the peroxidation of arachidonic acid micelles in an iron-catalyzed...

15/3,KWIC/69 (Item 5 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00224186
Protein-folding enzyme.
Protein faltendes Enzym.
Enzyme de pliage de proteines.
PATENT ASSIGNEE:
  REPLIGEN CORPORATION, (545550), 101 Binney Street, Cambridge Massachusetts 02142, (US), (applicant designated states: BE;CH;DE;FR;GB;IT;LI;NL;SE)
INVENTOR:
  Pigiet, Vincent P., 666 Main Street Apt. L-1, Winchester Massachusetts 01898, (US)

Rusche, James R., 21 Varnum Street, Worcester Massachusetts 01603, (US)
Schuster, Barbara J., 3520 Plaza Drive, State College Pennsylvania 16801, (US)
LEGAL REPRESENTATIVE:
Perry, Robert Edward et al , GILL JENNINGS & EVERY 53-64 Chancery Lane, London WC2A 1HN, (GB)
PATENT (CC, No, Kind, Date): EP 225156 A2 870610 (Basic)
EP 225156 A3 890111
APPLICATION (CC, No, Date): EP 86309188 861125;
PRIORITY (CC, No, Date): US 802569 851127; US 894421 860808
DESIGNATED STATES: BE; CH; DE; FR; GB; IT; LI; NL; SE
INTERNATIONAL PATENT CLASS: C12N-009/00; C12N-015/00; C12P-021/02; C07K-003/08;
ABSTRACT WORD COUNT: 70

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	452
SPEC A	(English)	EPABF1	5235
Total word count - document A			5687
Total word count - document B			0
Total word count - documents A + B			5687

...SPECIFICATION USA, 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. (1983) in "Thioredoxins...YM10 filter (Amicon, Danvers, MA). The buffer was exchanged with 50 mM Tris, 3 mM **EDTA** , pH 7.4 by diluting and concentrating the sample. The sample was stored at 4...enzyme in 0.1 M Tris, pH 7.4 or 9.0 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease or thioredoxin. At various times aliquots were assayed and...

...diluting the inactive RNase into 0.1 M Tris, pH 7.4 with 1 mM **EDTA** containing various amounts of thioredoxin shufflease and/or reduced DTT. At various times aliquots were...6X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate, 1 mM **EDTA**) and 10X Denhardt's solution (100 X - 2% bovine serum albumin, 2% ficoll, 2% polyvinyl...Reduced and Denatured RNase
At pH 9.0 (0.1 M Tris, 1.0 mM **EDTA**) thioredoxin shufflease or a mixture of thioredoxin shufflease and oxidized DTT increased the rate of ...

...denatured ribonuclease than is thioredoxin.
At pH 7.4 (0.1 M Tris, 1 mM **EDTA**) thioredoxin shufflease significantly increased the rate of refolding as compared to air oxidation. The time...

...said protein.
Example 18
At pH 8.5 (0.1 M Tris, 1.0 mM **EDTA**) thioredoxin shufflease increased the rate of reactivation of scrambled RNase as compared to air oxidation ...

15/3,KWIC/70 (Item 6 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00220520
Folding disulfide-cross-linkable proteins.
In Falten gelegte Proteine, durch Disulfid gebunden.
Proteines pliantes liees par pont disulfure en croix.
PATENT ASSIGNEE:
REPLIGEN CORPORATION, (545550), 101 Binney Street, Cambridge Massachusetts 02142, (US), (applicant designated states:

BE;CH;DE;FR;GB;IT;LI;NL;SE)
INVENTOR:
Pigiet, Vincent P., 666 Main Street Apt. L-1, Winchester Massachusetts 01898, (US)
Schuster, Barbara J., 3520 Plaza Drive, State College Pennsylvania 16801, (US)
LEGAL REPRESENTATIVE:
Perry, Robert Edward et al , GILL JENNINGS & EVERY 53-64 Chancery Lane, London WC2A 1HN, (GB)
PATENT (CC, No, Kind, Date): EP 208539 A2 870114 (Basic)
EP 208539 A3 880803
APPLICATION (CC, No, Date): EP 86305272 860708;
PRIORITY (CC, No, Date): US 753848 850711; US 812162 851223; US 859595 860505
DESIGNATED STATES: BE; CH; DE; FR; GB; IT; LI; NL; SE
INTERNATIONAL PATENT CLASS: C07K-015/00; C07K-015/12; C12P-021/00; C07K-007/00;
ABSTRACT WORD COUNT: 59

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	515
SPEC A	(English)	EPABF1	4071
Total word count - document A			4586
Total word count - document B			0
Total word count - documents A + B			4586

...SPECIFICATION USA, 75, 5827-5830). Other thioredoxin-like peptides include the class of seed proteins called **purothionins** that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. (1983) in "Thioredoxins...by diluting the reduced enzyme in 0.1M Tris, pH 7.4 with 1 mM **EDTA** containing various amounts of thioredoxin and/or oxidized DTT. At various times aliquots were assayed...

...diluting the reduced enzyme in 0.1 M Tris, pH 7.5 with 1 mM **EDTA** to a final concentration of 340 (mu)g/ml. Oxidized thioredoxin (100 (mu)M: was ...pH 7.5 or 0.05 M Tris, pH 9.0, each containing 1 mM **EDTA** . The regenerating system consisted of a catalytic amount of thioredoxin reductase and an excess (1...diluting the inactive RNase into 0.1 M Tris, pH 7.4 with 1 mM **EDTA** containing various amounts of oxidized thioredoxin preincubated with reduced DTT 30 min prior to addition...

?logoff hold
26feb05 10:16:39 User228206 Session D2370.5
$0.02 0.006 DialUnits File155
$0.21 1 Type(s) in Format 9
$0.21 1 Types
$0.23 Estimated cost File155
$3.71 0.629 DialUnits File654
$21.00 30 Type(s) in Format 3
$21.00 30 Types
$24.71 Estimated cost File654
$0.04 0.003 DialUnits File399
$0.04 Estimated cost File399
$2.39 0.504 DialUnits File349
$51.20 32 Type(s) in Format 3
$51.20 32 Types
$53.59 Estimated cost File349
$0.05 0.003 DialUnits File340
$0.05 Estimated cost File340
$0.03 0.006 DialUnits File5
$2.00 1 Type(s) in Format 3
$2.00 1 Types
$2.03 Estimated cost File5
$0.22 0.049 DialUnits File348
$10.20 6 Type(s) in Format 3
$10.20 6 Types